SURGERY

VOL. 44 JULY, 1958 No. 1

Society of University Surgeons

THE UNIVERSITY IN AMERICAN SURGERY

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THE UNIVERSITY AS A SURGICAL SITE

SINCE my theme deals with the role of the university in American surgery, it is quite fitting that we discuss the matter at a university that has grown up with the development of civilization in this hemisphere and at a medical school founded while the new nation was still struggling with the problems of federation versus union.

Although this university is old, it is not secure. We hope that Harvard has aged without senility, but it is much easier to speak of its past accomplishments than it is to prophesy the future. The future must depend upon the wisdom of the present generation and of those yet to come. Our concern over our future must be shared with the 84 university surgical services in this country that have come into being since those early days when John Warren held forth as our first Professor of Surgery.

Universities are not always a favorable place for cultural accomplishment. Shakespeare's plays were not written by a college professor, nor were Van Gogh's paintings the output of an art department. Literature, drama, the plastic and graphic arts have rarely thrived in the university atmosphere. Business, industry, and the engineering sciences have seldom found their directions pointed by university thought; the same may be said of the business schools. They supply men and teach skills rather than pointing new directions of effort.

Law and medicine bear a closer relationship to university activity. In the case of law, the legislative function upon which the practice of law is based is

Presidential Address delivered at the Annual Meeting of the Society of University Surgeons, Boston, Mass., Feb. 13-15, 1958.

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completely divorced from the university; it resides in government. So the law school serves largely as an interpreter and provides a basis for the maintenance of that equal justice under law which is the foundation of our society.

The profession of medicine is unique in the intimate and continuing relationship that exists between university activity and its cultural development. Yet even here the role of the university has waxed and waned, and we might well ask ourselves whether or not a university is now a favorable place for the development of surgery.

If we examine American surgery in the century between 1782, when Harvard Medical School was founded, and 1882, when Halsted was laying the foundations of his academic career in New York, we find comparatively little evidence of university leadership. Starting about 1880, university dominance began to be felt in surgery, as indeed it was in medicine as a whole. This can be traced to the application of laboratory methods in clinical investigation and to the work of Lister and Halsted. Since it is my conviction that research can occupy a distorted position in university surgery, we must admit right now that both Lister and Halsted did much research in their day. It was research that looked to a more effective and merciful care of the sick; it was clinical investigation. It has been the extension of this type of research, taking biologic science and bringing it to the bedside, that has moved surgery forward. The growth of surgery and its application to the intestinal tract, the cranial contents, the chest, the blood vessels, and the heart have come from clinical investigation in the universities, and these developments have revolutionized surgical practice. The period since 1880 has therefore brought the universities to a unique position of surgical leadership in American surgery. The fact is that we live in a period when universities are pointing the directions for American surgery; it has not always been that way and it may not always be that way in the future.

Despite these facts, it is not r e s e a r c h but the unity of surgery and its advance as a whole that is the objective of this Society. It is the sick patient in need of care, the research that helps that care, and the student learning from it, all taken together, that constitute university surgery. It is this unity for which this Society stands. It is not a bibliographic reference that is our focus, but a sick patient made well and a student made wise.

University surgery today is threatened by many disturbing trends. The most serious is that of the fragmentation or disjointing of its three major functions—teaching, practice, and investigation; each threatens now to go its independent way. It is our objective to keep them together. In terms of 1958 this means that we must learn to live with and discipline that unruly but brilliant child, research—while very assiduously keeping him fat and happy and in the family.

It is an easy matter for practice to leave the university environment; indeed, it always tends to. When practice is divorced from the teaching of surgery, the university becomes an arena where dry-as-dust academicians pursue their theories or clinicians perpetuate their prejudices. In surgery, such a ground is sterile and nothing grows.

By contrast, teaching never threatens to depart from the university. No one wants to teach the undergraduate except the university itself. Even there it is sometimes hard to find enough people to do it properly.

Unfortunately, research also tends to dissociate itself from teaching because research has become a channel for finance and for personal advancement to a greater extent than teaching and is more exciting and appealing as a focus for continued effort by the imaginative person. Research has become an end in itself rather than a means to discovery. It is the very eminence and productivity and promise of research that make it the most vulnerable point of university surgery today and a principal problem in the effectiveness and unity of the whole of university surgery. It is research that poses the problem in the years ahead, matters of balance and emphasis, of quality versus quantity, and of the relationships within the university departments of surgery.

Let us look first at those factors that lend strength and unity to the university establishment in surgery.

FACTORS OF STRENGTH AND UNITY IN UNIVERSITY SURGERY

The University in America.—A primary strength of university surgery stems from the position of the university itself in American Society. The university is a social microcosm that resembles the macrocosm in which it exists. James B. Conant, in his book, *The Citadel of Learning,* points out that the Western democracies are to be distinguished from the Eastern societies chiefly by the fact that the Western tradition is one of diversity, controversy, and tolerance whereas the Eastern system, epitomized in the Soviets, is one of standardization, conformity, and regulation. There can be no better definition of a university than a community of scholars who exhibit diversity, controversy, and tolerance. A university surgical service should exhibit the same qualities even though diversity, controversy, and tolerance may at times mean that the professor must submerge himself, favor the growth of other ideas, foster competition within his department, and share the spotlight with his colleagues. A professor who comes into a department, fears competition, and discharges or demotes all those who disagree or compete with him is not fostering the tradition of Western university life.

The Medical School in the University.—Just as the university stands strong in American society, so also the medical school stands strong in the university. Business, education, technical, and law schools are constantly threatened with vocationalism. One of their features which has raised medical schools far above the vocational level has been the fact—partly a coincidence—that the medical schools have become curators of human biology as well as professional schools. The medical schools of our great universities not only train doctors but are also storehouses of learning and of fundamental scientific advance in important fields of knowledge that bear on such diverse subjects as genetics, tropical hygiene, social psychiatry, child guidance, public health, communicable disease, and personnel selection—as well as medicine and surgery. This storehouse of human biology provides a basis for scientific advance and for education.

It gives the medical faculty a special position and a special strength among the graduate schools of the university. The medical school is truly a university within a university, with its own undergraduate school and its own postgraduate divisions.

Surgery in the Medical School.—Finally, of these three relationships, the place of surgery within the medical school is the most interesting and the most promising.

At this point a definition of surgery is essential. I like to define surgery as *an area of responsibility for the care of the sick.* It is not a way of doing things nor is it an act or procedure. It is defined in terms of direct responsibility for the care of the patient. Most surgical patients are suffering from diseases that are acute, focal, or traumatic, but these diseases do not lend themselves to any one all-inclusive definition. The surgeon is just as responsible for the care of acute pancreatitis as the physician is for the care of diabetic coma, even though both of them originate in the same organ and neither requires an operation. In most surgical responsibility, the most important single act is an open, anesthetized dissection, and it is the historic development of this method that has defined the area of responsibility. In the last 30 years it has become additionally clear that the surgeon, once having defined his large area of responsibility, has it all to himself. No one else can be relied upon to solve his problems, to help him, or even to understand his needs. The surgeon must consult with the biochemist, the physicist, the mechanic, or with anyone else he can. His reliance upon basic science is no more or no less than that of the physician, the pediatrician, or the psychiatrist, and no one can claim basic science as more truly his own partner than can the surgeon.

Surgery, like pediatrics, neurology, internal medicine, and psychiatry, grows in close relationship to the basic sciences. It is a common misnomer to state that some aspect—for example, the study of coagulation of the blood—is a "medical aspect of surgery." This is meaningless. It is a surgical aspect of surgery which, like many things in medicine, pediatrics, psychiatry, and all the other fields of human biology, requires knowledge of physical chemistry or protein interactions for its effective application. This contact with basic science is as much the property of surgery as it is of medicine, or any other field of responsibility.

When we emphasize the fact that surgery is defined as an area of responsibility for the care of the sick, then we can appreciate a little better the unique position of surgery within the medical school. About 60 per cent of the patients in a general hospital are taken care of by the surgical service. Sometimes this figure runs as high as 80 per cent. The average medical student sees more of the regular daily practice of the care of the sick on a surgical service than he does in any other division. In the mind of many a medical student today, the great physician he sees is not a physician at all but a surgeon. This is merely due to the fact that in a general hospital, which is the site of teaching, surgery is actually practiced. Medicine, pediatrics, and psychiatry are increasingly practiced somewhere else: the home, the office, or the special hospital.

As internal medicine grows in effectiveness, fewer and fewer patients need to be admitted to a hospital. The departmental concern with basic science then becomes progessively greater. This is fascinating and important but hardly inspiring to the medical student, who seeks inspiration from the merciful wise physician as he cares for the patient.

As surgery grows in effectiveness, more and more patients are admitted to the hospital for direct, individual, personal care—patients who otherwise would have been given up as hopeless or who would have been relegated to a life of palliative medication. If the student goes on rounds in a major urban hospital with a first-rate practitioner of surgery one day and with the professor of surgery the next, he sees similar patients handled in much the same way. By contrast, the university pediatrician, dealing with highly specialized pathologic cases on his wards, has a patient load which hardly resembles that of his practicing fellow who deals largely with the important guidance of well children, care of worried mothers, and respiratory infection, in home and office. This same dichotomy is increasingly true of internal medicine as office practice differs from that of the hospital.

The advance of surgical science has thus brought the practice of the university and the universe of the practitioner closer together rather than farther apart. This unity of practice and of study is one of the great strengths of university surgery. It should be recognized, fostered, and capitalized upon—but not exploited. This special concern for an individual patient's welfare is the greatest heritage of surgery, and it should be greeted by the university, not thrust aside.

Youth and Research.—The last of the many strengths of university surgery that I wish to discuss here has to do with youth. When responsibility for surgical care is given to young men, the result is advance. Older people have heavily entrenched habits. At some age, probably about the time of retirement from active membership in this Society, the surgeon starts to say on rounds, "This is how I do it," rather than, "This is why I do it." The residency system and clinical research in surgery are thus very closely related and mutually dependent. This is a point that many foreign visitors never understand about American surgery. Research born in the minds of young men is good research. It is appropriate research. It is living research. It has been responsible for the eminence of American university surgery today. It is not the research executive or the department head, busily operating upon private patients or filling out forms and applications, but instead the actual ideas in the minds of the younger people that count. The university service joins young men and sick patients together in a proper relationship in which the student can participate. This holy alliance is responsible for the eminence of American university surgery today.

That this Society is a young man's society is of course a by-product of this same American trend. While it is a young man's society, we should note well that as a group our younger generation is not so young as before. Many of our founders and those elected in the first 4 years of the Society's life were active in the Society for 15 to 18 years. Now the residency system, the military, the

academic pursuit, all take precious years and the average age of attaining membership is about 37. This means that the average duration of activity in the Society will be but 8 years. The interval between the age of the "bright young man" and the age of retirement from active membership is getting shorter and shorter! We must look at this problem and seek a solution before the duration of activity in the Society is reduced to absurdity.

FACTORS OF WEAKNESS AND DISUNITY IN AMERICAN SURGERY

Research: Quality vs. Quantity.—Turning now to some of the factors that threaten this unity of university surgery and weaken it, let us look first at some of the abuses of research. The number and nature of publications and the number of journals in which they can be published indicate the breadth and the lack of depth of present-day research. University research, in order to survive, must be small and perfect. The emphasis must be on quality, not quantity. Good surgical research is personal, done by a thinking man. It cannot be turned out by a busy practitioner between operations or by a statistical machine, nor can it be turned out by a staff of trained secretaries in a follow-up clinic, or by a group of chemical technicians, or by a group of editorial librarians who publish a bibiography of two or three hundred references that the author has never seen.

The emphasis must be on thrifty quality, not wastefully diffuse mediocrity. If the universities do not maintain these standards, the plum of research will fall from their hands into the hands of others who are very happy to receive it, and the unity of university surgery, as we have known it, will cease to exist. The platitude "publish or perish" was coined to describe the academic environment in which publication, no matter of what, was the sine qua non for personal advance. This is a real hazard in surgery. One year of research with a 10-minute paper in October does not create a university surgeon.

Research: Too Basic?—Besides becoming too big and too mediocre, surgical research can also become too basic. This may seem a heterodox view in an age when the emphasis is on basic science, but a surgical department is, generally speaking, not the place for basic science. There are other departments that can do it better. There are too many important things to do in the very urgent and elegant work of applied science. In the trend to so-called basic science, it is all too easy to forget that somebody must bring the basic science to the clinical problem, and it is this that has advanced surgery. Good clinical investigation is the touchstone of good surgical research now, just as it was with Lister and Halsted. The surgical investigator must be a bridgetender, channeling knowledge from biologic science to the patient's bedside and back again. He traces his origin from both ends of the bridge. He is thus a bastard, and is called this by everybody. Those at one end of the bridge say he is not a very good scientist, and those at the other say he does not spend enough time in the operating room. If only he is willing to live with this abuse, he can continue to do his job effectively. It is much harder to stay in the middle of the bridge than it is to retreat to one end or the other. If he gets scared and retreats to one end of the bridge,

he becomes a practicing surgeon. If he retreats to the other end of the bridge, he becomes an investigator trying to live the life of a biologic scientist. In either event, he loses his stature and his function in advancing surgery.

To understand biologic science and bring it to the bedside, the surgeon must study and know the concepts and techniques of basic science. They must be a part of him and a part of his education. But they should not take over as his only focus. As the young surgeon meets biologic science, he should be charmed, not seduced—intrigued, but not captured. He may dwell with her for a while but should not settle down with her for life.

I said that research tends to dissociate itself from teaching. Not only is this true in the career of the individual but also in the structure of the hospital and the balance of the curriculum. It is much easier to raise money to build a research building than it is to finance a new associate professorship. Once the building is built, students do not have a place in it; as hospital research becomes progressively more basic, the student sees less and less of it. This is not true of good clinical investigation, in which the student participates.

Research: Where and by Whom?—I mentioned before that if the universities do not do a good job for surgical research, others will be there ready to grab the plum. There are many institutions in this country other than the universities happily waiting to do surgical research. In the past 5 years they have suddenly discovered that research means money and prestige as well as isolated advance and that it need not be the sole prerogative of the university. These institutions range from the great private clinics, who now employ full-time investigators to keep the fire burning, through some of the federal hospitals, on down to units who have discovered that with 2 laboratory benches, a pH meter, and 3 dogs in a cage, they can call it a foundation, get the application forms, and they are in business—that is, so long as they can pass the "written."

The universities will do well to realize that these institutions can easily outdistance them when it comes to sheer, pure, unadulterated research. The independent foundation and the research group have no problems of teaching, no students, no faculties, no faculty meetings, no interns, no residents, and I assume that they have not yet discovered that great device, the committee. What patients there are are cared for by another team or second platoon who are wholly involved with clinical work so that the foundation can concern itself 100 per cent with research. These institutions can conduct research from one end of the year to another, with never a student entering the door nor a patient within earshot. This makes quite a contrast with the surgical services represented by this Society where the student, the patient, and the surgeon rub elbows with one another and enrich one another's work and welfare.

Surgical advance has come when the clinical investigator has brought the fruits of scientific research to the patient; this cannot be done effectively if the care of patients is divorced from clinical investigation. Surgery evidently makes the most yardage with a one-platoon system.

On Understanding Surgeons: The Egghead.—The university approach to surgery is not always easy for other people to understand. University surgery stands for intellectualism. The "long-haired" surgeon—the egghead—is the university surgeon. Intellectualism in surgery is mildly distasteful to the physician. By this view the surgeon should operate when told to, do his work, and shut up; if he is going to study anything, he should concentrate on the operative mortality. The thinking, questioning, "long-haired" surgeon represents a mild threat to the leadership, kudos, and knowledgeable position of others on the faculty. This lack of understanding takes the form of demoted curriculum, second-rate appointments, and lack of space and support, and it leads to a complete divorce between practice and study, because one of the by-products of failure to understand university surgery is to see to it that all the practice of surgery is done by someone else. This is a trend that we might call "antirationalism."

The best cure for antirationalism is to prove the practical effectiveness of rationalism. It has been said of physics that "after Sputnik, the egghead rides again." In surgery, by the same token, "after the asystolic open heart, the egghead rides again."

The surgeon has often been called a stupid, cutting man. Let us be thankful that we have been given a high calling in the care of the sick and let us deal with lack of understanding by strengthening university departments and demonstrating to the rest of the faculty that the best cure for the historic faults of surgery is a well-balanced and effective department, doing effective research and teaching to advance the care of the sick.

The Schizoid Professor.—Finally, from this catalogue of problems facing university surgery, I would like to mention the career pattern of the people generally designated as the professors who run the departments. Increasingly, professorial appointments are made as an award for merit. An active and productive person is crowned with a laurel wreath, thus revealed for general acclaim, and promptly snowed under with the work of a department. Despite this shortcoming, I think there is good evidence to support the concept that young people make good leaders and that the trend should be continued. But notice this: these young men, once they are made professors, must see to it—despite the laurel wreath and the snowstorm—that their own work and their own intellectual growth continue, so that 10 years later they will be running a department of surgery and not a correspondance factory. This does not mean that they need be selfish or intolerant. Diversity, controversy, and tolerance actually thrive in a department where the professor is attending to his business and attending to it well. For the professor to continue with his own work is essential to the life of the department and will, in the long run, be the central focus of continuing growth within the department.

One answer to the problem of departmental administration snowing under the professor has been the creation of the split professorship. One person does

the clinical work, and another the research and paper work. There is an interesting analogy here to foreign policy. It has often been said that foreign policy only becomes definable in retrospect. Then it is seen to be not so much a plan as a sum total or net vector of all the individual acts that were done. The same thing is true of running a university department of surgery. It is hard to tell just where it is going at any one moment. The net lifetime policy can be analyzed only in terms of the net vector of millions of individual acts. Most of these acts are carried out not on the wards, or the operating room, or outpatient department but, sad though it is to relate, in arranging the appointments, finance, laboratory, and, most important of all, naming and keeping personnel. This very "dirty-work" (which would be so appealing to turn over to somebody else) actually in its final analysis represents the policy-making of the department today. It can truly be said that the man who operates on the patients and teaches the students and the man who answers the mail must all be the same man if the department is to push forward in any direction at all.

THE FUTURE: BALANCE IN SURGERY

Looking to the future, then, how can we balance off these weaknesses with these strengths? As Lord Cecil said in his life of Conrad: "What one lives for may be uncertain but how one lives is not." The university surgeon must first be a good surgeon. How he lives finds its most important expression in the care of the sick, not necessarily large numbers of sick but effective care for those to whom he directs his attention, and they must never be prostituted or exploited for his own advancement either fiscal, scientific, or academic. "The purposes of teaching and the techniques of research acquire importance only when they are related to the care of patients . . . and only where the care of patients is conducted with wisdom and mercy will teaching and research also thrive."*

If research sometimes seems overemphasized, distorted, sloppy, diffuse, mediocre, ill-founded, ill-conceived, and ill-read, then what, in the future growth of American surgery, should balance it out? The best counterbalance for a lot of poor research is a small amount of excellent research. And make no mistake, much worse than mediocre research is no research at all. In 1912, Halsted wrote, "We want men to have learned to work for work's sake and who find in it and in the search for truth, their greatest reward. . . . Students ape their teachers. The type of man who stimulates investigation is the best." So research must be there, but it must be good.

In judging the quality of research, I have found it best to use three criteria: originality, productivity, and significance. It is the man who studies a few things, picks them well, and finds and publishes important answers who deserves recognition. In addition to these well-known facts, one must somehow recognize a man's devotion, constancy of purpose, and his meaning to the total university activity. And here we see a change in the history of this Society. In the past 10 years, membership in surgical societies has increasingly been

*"Principles and Procedures," Peter Bent Brigham Hospital, Surgical Service, Boston, 1953.

based upon academic productivity, measured in its crudest form by bibliographic volume without regard to originality or significance. I hope this has not occurred in the Society of University Surgeons, but I am afraid that we are drifting in that direction. Yet, if we look at the members of this Society, we find several of the most eminent whose chief contribution to surgery has lain, not in a potpourri of papers on a hash of subjects, but instead in their single-minded devotion to the growth of an important field of learning in surgery, the objectives of the university, their effectiveness in the care of the sick, their interest and activity in teaching, and the fact that they sacrificed other more lucrative pursuits in order to foster the growth of a department and a field of knowledge.

SUMMARY

University surgery is in a remarkably strong position in America today. It has been that way for only about 50 years and it may not be that way in the future. It is strong because it has contributed to the practice of surgery through the effective application of biologic science. That strength has matured in the happy home environment of student teaching and merciful patient care. It is this brilliant but unruly child, research, who today threatens to stir up the house and break up the connection. University surgery must maintain its balance, its status, and its dignity as a healthy family of good care and effective teaching so that research may thrive in the hands and minds of young men. The research must then be long on quality even if short on quantity; it is a prime responsibility of American university surgery and of this Society to set and maintain the highest possible standards of originality, productivity, and significance in clinical investigation so that the unique advance of American surgery under the leadership of its universities may continue in the future as it has in the past.

AMINO ACID THERAPY OF AMMONIA INTOXICATION

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THE treatment of ammonia intoxication includes methods to control the gastrointestinal production of ammonia as well as measures to enhance the rate of removal of ammonia from the blood. The production of ammonia in the gastrointestinal tract is regulated by limitation of the intake of protein,[34] control of gastrointestinal bleeding, prompt removal by catharsis or lavage of blood that may have accumulated in the gastrointestinal tract,[22, 38] and oral administration of antibiotics to diminish the numbers and activity of the intestinal bacteria that produce ammonia from nitrogenous substrates.[9, 33]

Although control of the gastrointestinal source of ammonia is an essential component of the therapeutic regimen, it is equally important to attempt to remove the ammonia that continues to accumulate in the blood. In an effort to reduce high concentrations of ammonia in the blood, attempts have been made to enhance the reactions whereby ammonia is normally detoxified in the body. This has been accomplished experimentally by the administration of the amino acids which are essential for the two major biochemical reactions for disposal of ammonia, that is, transamination or the production of urea.

The transfer of amino groups between glutamic or aspartic acid and certain keto-acids such as alpha-keto glutaric acid, is catalyzed by specific enzymes known as transaminases.[2] Conversion of glutamic acid to its monamide glutamine involves the acceptance of a mole of ammonia (Fig. 1). This reaction was first demonstrated by Krebs[17] and Weil-Malherbe[37] in brain. Glutamine formation from glutamic acid is now known to occur in many tissues other than brain. In fact, transaminases have been found in all tissues of the body except bone; the highest concentrations are in heart muscle, skeletal muscle, brain, liver, kidneys, testes, and lung, in order of decreasing concentration. The removal of ammonia by peripheral tissues has been demonstrated by the findings of significant differences in the arterial and venous blood ammonia concentration when the blood ammonia is high.[1] Transamination reactions account for the removal of ammonia by the peripheral tissues.

Because of the ability of glutamic acid to detoxify ammonia by the formation of glutamine, Sapirstein[32] suggested its use as a therapeutic agent for ammonia intoxication. He found that glutamic acid protected rabbits from the convulsions induced by the intravenous infusion of ammonium chloride. Although aspartic acid may remove ammonia from the blood in a similar manner

Supported by the National Institute of Arthritis and Metabolic Diseases (Grant A-1053) and the Christine Breon Fund for Medical Research.

Read at the Annual Meeting of the Society of University Surgeons, Boston, Mass., Feb. 13-15, 1958.

by the formation of asparagin, it was found to be ineffective in preventing experimental ammonia intoxication under the conditions of Sapirstein's experiments.

In experiments in which isotopic amino acids were given intravenously, glutamic acid did not penetrate the blood-brain barrier.[10] Although high concentrations of the isotopically labeled glutamic acid were found in the liver, intestines and kidney, none was recovered from the brain. From this evidence it appears that the effect of glutamic acid on ammonia intoxication can be attributed to its role in the peripheral detoxification of ammonia rather than to any direct effect within the brain itself.

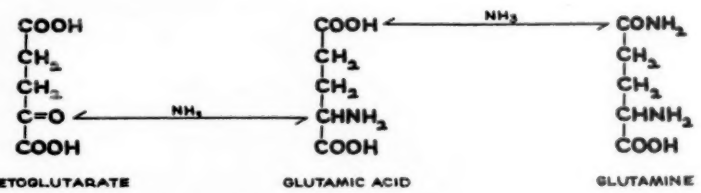

Fig. 1.—Diagram of the biochemical reaction whereby ammonia is detoxified by glutamic acid. In this transamination reaction a mole of ammonia is accepted when glutamine is formed from glutamic acid or when glutamic acid is formed from alpha-keto glutaric acid. These reactions are catalyzed by the enzyme transaminase and are reversible.

The treatment with glutamic acid of ammonia intoxication associated with clinical disease was first reported by Walshe.[35] Although he did not measure the blood ammonia it was his opinion that glutamic acid influenced the clinical improvement of 3 patients with subacute or chronic liver disease. This report stimulated several other studies on the use of glutamate for the treatment of ammonia intoxication.[4, 8, 15, 23, 36] Although these reports have varied with regard to the success or failure of this therapeutic agent, the weight of evidence seems to indicate that the administration of monosodium glutamate in adequate doses is frequently followed by a decrease in the blood ammonia. However, in severe cases of ammonia intoxication, the reduction in the concentration of ammonia in the blood appears to be transient.[21] This may be due to the fact that the detoxification reaction, which involves conversion of glutamate to glutamine, rapidly becomes saturated. A reversal of the reaction may then occur, so that ammonia is actually produced from glutamine. This may explain the observation[8] that, although a temporary decrease in blood ammonia occurs following glutamate therapy, a secondary increase which actually exceeds the preinfusion blood ammonia concentrations may occur.

The formation of urea is the other important reaction involved in the detoxification of ammonia within the body. This reaction, which is quantitatively more important than transamination, is believed to occur only in the liver. The description of the mechanism of urea production as originally given by Krebs and Henseleit[18] as a result of experiments with slices of liver has recently been expanded by Ratner and associates.[30, 31] The introduction of carbon dioxide and of ammonia into the cycle probably occurs by a process of transcarbamylation with a recently discovered "activated" compound, carbamyl phosphate (Fig. 2).[16]

In a number of experiments the amino acid arginine was shown to affect the metabolism of ammonia by enhancing urea production within the liver. In these experiments[13, 24, 25] it was found that the rapid increase in blood ammonia that occurred after giving amino acids intravenously could be entirely prevented by the administration of arginine. This was so whether a single amino acid such as glycine or an amino acid mixture such as casein hydrolysate was used to cause the increased concentration of ammonia in the blood. Greenstein and his co-workers[3, 11] also demonstrated the protective effect of arginine against ammonia intoxication which was induced by the intraperitoneal administration of ammonium acetate to rats. They also showed that this effect was associated with a decrease in blood ammonia concentrations and an increase in blood urea nitrogen.

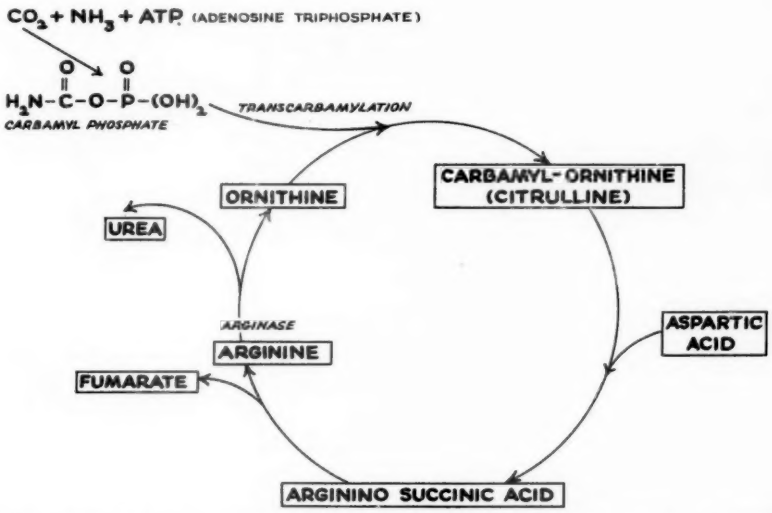

Fig. 2.—Diagram of the essential components of the urea cycle. Carbon dioxide and ammonia, in the presence of adenosine triphosphate, enter the cycle as carbamyl phosphate which combines with ornithine. Through a reaction of transcarbamylation, carbamyl-ornithine (citrulline) is formed. An additional mole of ammonia is added to the cycle when aspartic acid combines with carbamyl-ornithine to form argininosuccinic acid. Fumarate is then separated from argininosuccinic acid to form arginine which through the action of the enzyme arginase forms urea and ornithine. Thus with each completion of this cycle two moles of ammonia are removed with formation of each mole of urea.

A clinical evaluation of the effectiveness of arginine for the treatment of ammonia intoxication in 15 patients with increased blood ammonia concentrations that were associated with various degrees of encephalopathy secondary to a variety of clinical situations was first reported in 1956.[26] When arginine therapy was used in association with other measures for the treatment of ammonia intoxication there was a prompt reduction of the ammonia in the blood, and this was associated with clinical improvement which in some cases occurred promptly and in others was delayed for several hours.

Several additional clinical reports have appeared confirming the ability of arginine to decrease the blood ammonia concentrations in patients with ammonia intoxication.[7, 20, 21, 27, 28] An exception is the report of Fahey,[6] who was unable to demonstrate any measurable effect on the blood ammonia concentrations when arginine was administered intravenously to 8 patients with spontaneous hepatic encephalopathy.. Seven of these patients were apparently

moribund with neoplastic disease in the liver. The eighth patient, who had cirrhosis of the liver with hepatic failure, died 9 days later in coma without any discernible precipitating cause. It is apparent that any therapeutic regimen involving a metabolic reaction by an organ must ultimately fail when the function of that organ becomes irreversibly damaged.

It is evident that both glutamate and arginine can lower the blood ammonia and that each amino acid does so by a different metabolic reaction that varies both in the promptness and duration of its effect. Glutamate appears to react with ammonia more rapidly than does arginine but there is evidence that this reaction may become saturated so that in the presence of a continuing rapid rate of ammonia production, this amino acid may no longer be effective. The action of arginine, while somewhat slower, is more permanently effective because urea formation, the biochemical reaction which it enhances, is well adapted to the removal of large quantities of nitrogen over a longer period. These considerations have indicated that both amino acids should be used in the treatment of ammonia intoxication. Recently the compound arginine (argininium) glutamate has been made available for experimental and clinical investigations. This compound supplies both amino acids in approximately equal quantities. It is a white, crystalline powder approximately 40 per cent soluble in water, with a pH of 7.0 in a 10 per cent solution. The use of this compound obviates the introduction of sodium ions which must necessarily be given in connection with glutamic acid treatment because of the low solubility of the free acid.

In a series of experiments in which ammonia intoxication was induced by the intravenous administration of glycine, the effect on the blood ammonia of the addition of monosodium glutamate, arginine, or arginine glutamate was observed (Fig. 3). Both arginine and arginine glutamate effectively prevented the increase of ammonia in the blood produced by the infusion of glycine alone; monosodium glutamate was only moderately effective. In order to evaluate the contribution of the arginine component of arginine glutamate, the effect of a quantity of arginine equivalent to that contained in the dose of arginine previously used was studied. It was apparent (Fig. 4) that arginine alone was almost as effective in preventing an increase in the blood ammonia after glycine infusion as a quantity of arginine glutamate containing the equivalent amount of arginine.

The variations in clinical response to amino acid therapy of ammonia intoxication are illustrated by this brief summary of the results of therapy* in 90 patients grouped according to the 5 etiologic classifications of ammonia intoxication to be described.

I. Patients With Ammonia Intoxication of Exogenous Origin.—These 14 patients developed ammonia intoxication from the oral ingestion of ammonium salts or Diamox used as diuretic agents or from ammonium salts given intravenously to correct alkalosis. No deaths occurred in these 14 patients, 11 of whom were treated with arginine. Clearing of the sensorium associated with

*Detailed clinical histories and biochemical results of treatment in many of these cases have been published elsewhere.[27]

a reduction of the blood ammonia occurred in each case in less than 5 hours after treatment with arginine. Clinical and biochemical evidence of improvement was slower in patients who did not receive arginine therapy. Although it appears that the recovery of these patients was accelerated by treatment with

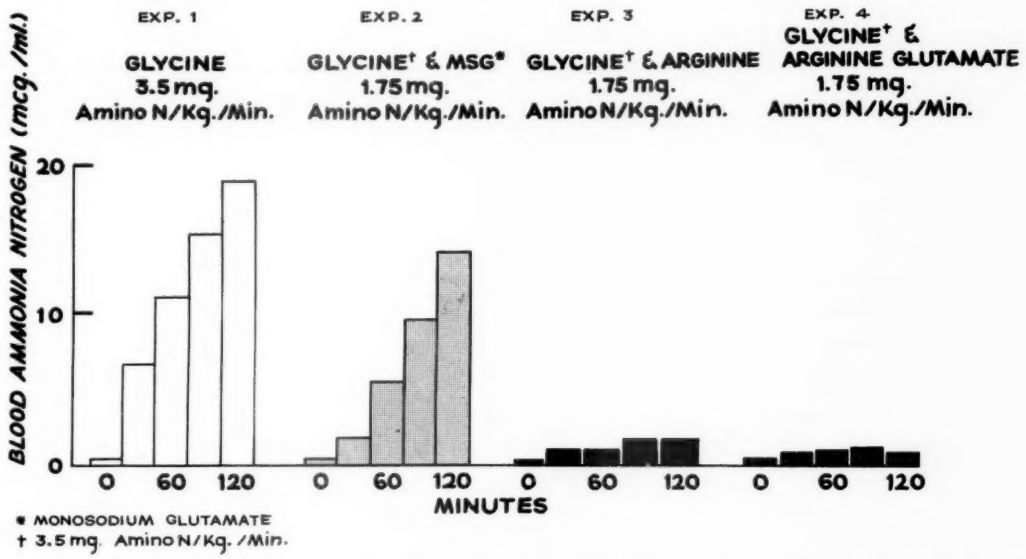

Fig. 3.—Graph showing blood ammonia concentrations in dogs, comparing infusion of glycine alone with infusions of glycine in combination with monosodium glutamate, arginine, or arginine glutamate. Arginine and arginine glutamate effectively protected animals from experimental ammonia intoxication produced by the rapid infusion of glycine, whereas monosodium glutamate had only a moderately protective effect.

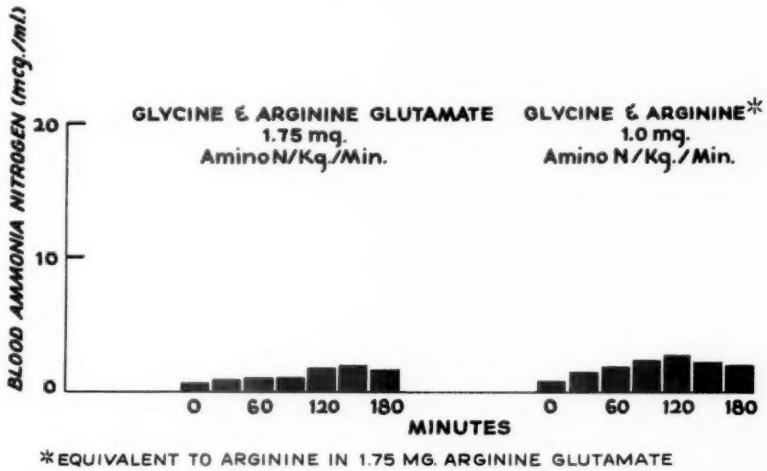

Fig. 4.—Graph showing the effect on the blood ammonia concentrations of intravenous infusions of glycine in combination with either arginine or arginine glutamate in equivalent quantities. The effect of arginine glutamate in protecting experimental animals from the ammonia intoxication resulting from infusion of glycine is primarily due to the arginine component of arginine glutamate.

arginine, it is likely that prompt recognition of the cause of the patient's ammonia intoxication followed by the withdrawal of the toxic agent would have resulted in the recovery of most of them.

II. Patients With Portal Cirrhosis and Gastrointestinal Hemorrhage.—In this group of 48 patients with chronic liver diseases, ammonia intoxication occurred as a result of an increase in nitrogenous substances within the intestine secondary to gastrointestinal hemorrhage. In 30 of the patients, 28 of whom were treated with arginine, bleeding was due to esophageal and gastric varices, and in the other 18 patients bleeding was secondary to peptic ulceration. Fourteen deaths, a mortality of 47 per cent, occurred in the group of patients with esophageal bleeding. Although the mortality rate was high, death was usually due to failure to control the bleeding rather than to "hepatic coma." In the group of 18 patients with bleeding other than from varices, 17 were treated with arginine, with only 2 deaths.

This was the most difficult group of patients to treat. In addition to the excessive quantities of ammonia produced by the action of bacteria on the large amounts of blood in the intestine and the probable further reduction of hepatic function due to shock[14, 29] and hepatic ischemia,[19] it is also possible that there might have been an arginine deficiency in these severely nutritionally debilitated patients.* The fasting blood concentration of arginine in the serums of 9 patients with portal cirrhosis and bleeding esophageal varices who were either in stupor or coma when first examined is shown in Table I. In all of these cases the serum arginine concentrations were below the lowest concentrations reported as normal.[12]

TABLE I. SERUM ARGININE CONCENTRATIONS IN PATIENTS WITH PORTAL CIRRHOSIS AND HEMORRHAGE

PATIENT	SEX	AGE	SERUM ARGININE (MG./100 ML.)
J. H.	M	71	0.6
B. D.	F	58	0.7
T. B.	M	63	0.9
R. K.	M	62	0.7
B. R.	M	66	0.8
G. A.	F	48	0.7
F. W.	M	54	1.0
P. D.	F	51	0.9
M. A.	M	65	0.8
Average of 9 patients			0.78
Normal serum arginine			1.2 to 3.0

III. Patients With Surgical Shunts.—This group of 16 patients with ammonia intoxication includes 7 who had portacaval shunts and 9 who had splenorenal shunts for relief of portal hypertension secondary to intra- or extrahepatic portal obstruction. Fourteen were given arginine. Two of these 14 died. Because of the large shunt around the portal venous system of the liver in all of these patients, the blood ammonia increased rapidly, and particularly so after a high protein meal. However, the results of treatment of hepatic coma in these patients were usually successful if the cause of their symptoms was promptly recognized.

*Fahey[5] observed that arginine-free amino acid preparations when given intravenously were more toxic to patients that had been on a low-arginine diet prior to the infusion. He also reported that an increase in blood ammonia following infusions of amino acids could be more easily induced in fasting normal subjects.

IV. Patients With Acute Hepatic Insufficiency.—This group of 9 patients had acute hepatic failure with ammonia intoxication as a result of viral hepatitis or ingestion of hepatotoxins. All were treated with arginine. Only 2 deaths occurred in this group. In these 9 patients the increase in blood ammonia, although usually moderate, was nevertheless toxic, because it occurred rapidly in patients not previously subjected to high concentrations of ammonia in the peripheral blood. Ammonia intoxication was readily controlled as soon as regeneration of the liver occurred, but it was a serious complication during the early stages of acute hepatic insufficiency. For this reason the treatment of ammonia intoxication was an important part of the early management of these patients.

V. Patients With Chronic Hepatic Insufficiency.—This group includes patients who had severe hepatic tissue destruction secondary to long-standing cirrhosis of the liver or extensive replacement of the hepatic parenchyma by neoplastic disease. In all of these patients, coma accompanied by increased blood ammonia concentration occurred spontaneously without an apparent precipitating cause. The degree of hepatic failure in these cases was apparent from the concomitant occurrence of a marked hypoalbuminemia with ascites, rapidly increasing jaundice, and a decrease in prothrombin concentration with a hemorrhagic diathesis that was completely unresponsive to treatment with parenterally administered vitamin K. In such cases, hepatic function had obviously been reduced to such a critically low level that no therapeutic maneuver could be expected to be successful.

In a series of 13 such patients, 11 died even though 9 of them were treated with arginine. Following arginine therapy the blood ammonia decreased slowly but increased again soon after cessation of treatment with arginine.

The over-all mortality rate for all of the 5 groups of patients described was 32 per cent (29 of 90 patients). However, 16 of these deaths occurred in patients with cirrhosis of the liver with hemorrhage (Group II) and 9 were patients with chronic hepatic insufficiency (Group V). The results of the treatment with arginine of 90 patients with ammonia intoxication associated with various degrees of encephalopathy are summarized in Table II.

TABLE II. RESULTS OF ARGININE THERAPY IN AMMONIA INTOXICATION
(90 Patients)

ETIOLOGIC CLASSIFICATION	NUMBER OF	
	CASES	DEATHS
I. Exogenous ammonia intoxication	11	0
II. Portal cirrhosis with hemorrhage:		
Bleeding esophageal varices	28	14
Other causes of bleeding	17	2
III. Surgical shunts:		
Portacaval anastomosis	6	1
Splenorenal anastomosis	8	1
IV. Acute hepatic insufficiency	9	2
V. Chronic hepatic insufficiency	11	9
Total	90	29

It has often been difficult to correlate the reduction of blood ammonia with the clinical improvement of patients with hepatic encephalopathy due to ammonia intoxication. This lack of correlation between the blood ammonia concentrations and the severity of symptoms has been responsible for conflicting reports on the etiologic significance of increased ammonia concentrations in

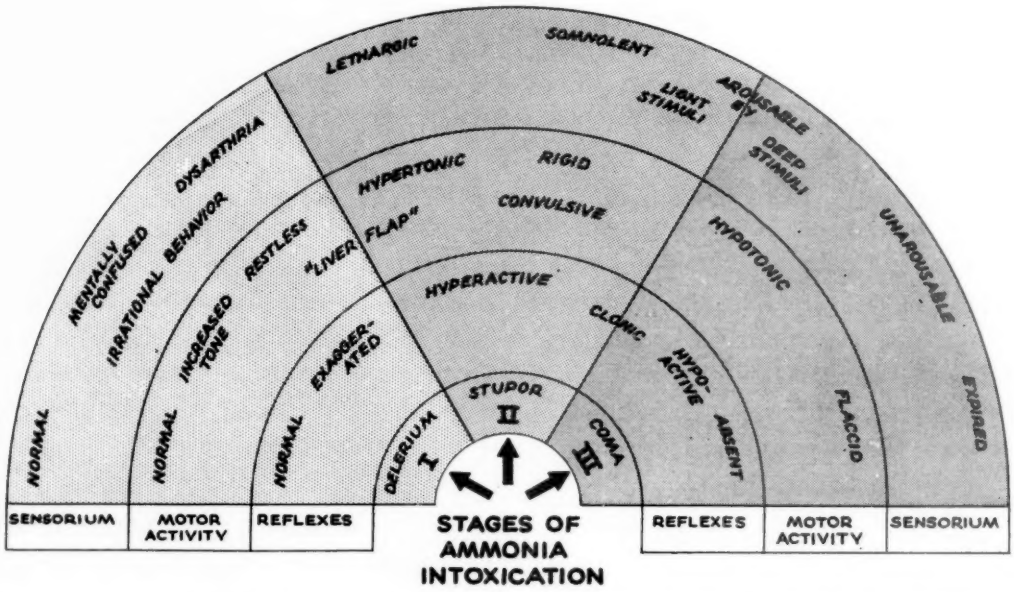

Fig. 5.—Diagram illustrating the various neurologic manifestations designated as "stages" of ammonia intoxication.

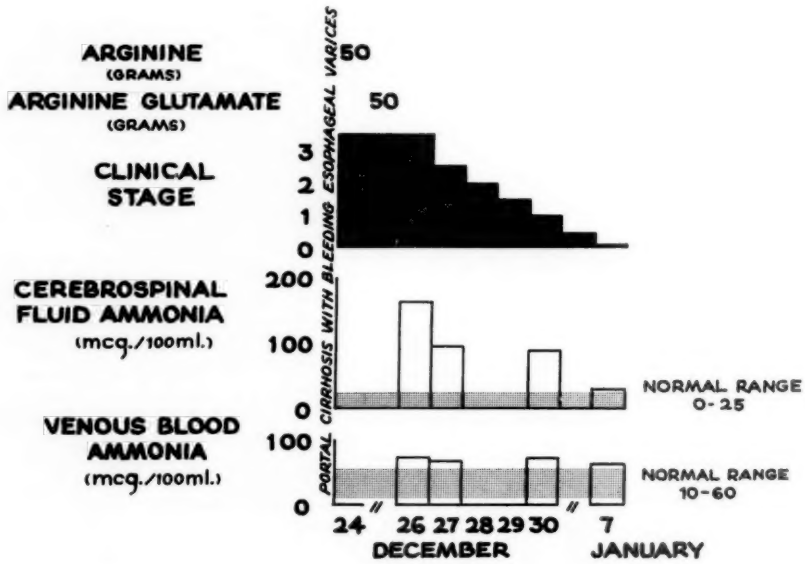

Fig. 6.—A comparison of the blood and cerebrospinal fluid ammonia concentrations in a patient with "hepatic coma" and ammonia intoxication caused by bleeding esophogeal varices associated with Laennec's cirrhosis. In this patient it appears that the clinical stage of "hepatic coma" was more closely correlated with the concentration of ammonia in the cerebrospinal fluid than that in the peripheral venous blood.

"hepatic coma." A delay in clinical improvement usually follows the therapeutic reduction of the blood ammonia concentrations. It has been observed in this laboratory that this delay was directly related to the duration of the ammonia intoxication, that is, patients who had been intoxicated with ammonia over long periods responded slowly to therapy. A probable cause for this delay in the response to treatment of hepatic encephalopathy is illustrated by a comparison of the blood and cerebrospinal fluid ammonias during various clinical stages (Fig. 5) of ammonia intoxication in a patient with "hepatic coma" caused by bleeding esophageal varices associated with Laennec's cirrhosis (Fig. 6). It is apparent that coma persisted even though the concentration of ammonia in the blood was reduced to normal. However, the concentration of ammonia in the cerebrospinal fluid remained high, and improvement of the patient's neurologic condition did not occur until the ammonia in the cerebrospinal fluid was reduced. This indicates that the clinical stages of hepatic encephalopathy may be more closely correlated with the concentration of ammonia in the cerebrospinal fluid than with the blood ammonia concentration and may explain the delay in clinical improvement following therapeutic reduction in concentrations of ammonia in the blood of patients with "hepatic coma."

SUMMARY

Methods to reduce increased concentrations of ammonia in the blood have been directed toward enhancing the reactions whereby ammonia is normally detoxified in the body. The rationale for the therapeutic use of amino acids for the control of ammonia intoxication is based upon the metabolic reactions of transamination (with glutamic acid) and the synthesis of urea (with arginine). Because each amino acid removes ammonia by a different metabolic reaction, the use of a compound containing both amino acids was investigated experimentally to determine whether it would be more effective than either amino acid alone. This compound, arginine glutamate, was found to be only slightly more effective in reducing the blood ammonia than was arginine in equivalent doses.

Arginine was used for the treatment of ammonia intoxication in 90 patients. A reduction in the blood ammonia concentrations of these patients was followed usually by improvement in their clinical status. The over-all mortality rate was 32 per cent.

A possible explanation for the delay in clinical improvement that occurs following reduction of blood ammonia concentrations in patients with ammonia intoxication may be found in the observations made on a patient in whom improvement in the sensorium was found to be more closely correlated with the concentration of ammonia in the cerebrospinal fluid than with the concentration of ammonia in the venous blood.

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(For discussion, see page 30.)

INFLOW AND OUTFLOW CHANGES IN AMMONIA CONCENTRATIONS OF LIVER, MUSCLE, AND BRAIN

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DURING the past few years the studies of many investigators have added to the knowledge of the behavior and metabolism of ammonia.[2, 4, 5, 7, 10] In an attempt to define more clearly the basic responses of ammonia, varying amounts of ammonium acetate were infused into dogs and the levels of ammonia determined on samples taken simultaneously from various regions.

METHODS

The animals used were mongrel dogs selected randomly. All dogs under study were anesthetized with intravenous sodium pentobarbital. Polyethylene catheters were placed in the hepatic and portal veins in order to obtain samples for determining the relative amounts of ammonia cleared by the liver (Fig. 1). Through a right rectus incision a polyethylene catheter was inserted into a large proximal mesenteric vein and pushed forward until its tip was in the portal vein. A small polyethylene catheter was anchored in a distal vein in the mesentery of the small intestine for the purpose of infusing the ammonium acetate solution. A long ureteral catheter was inserted through the left jugular vein into the inferior vena cava and passed into the hepatic vein under fluoroscopic observation. Infusions of 5 per cent glucose in water were allowed to drip slowly into the catheters of the hepatic and portal veins in order to maintain their patency for sampling. In some animals the right carotid artery and right jugular vein along with the left femoral artery and femoral vein were exposed surgically for sampling.

Blood pressures were measured by the direct manometric method with a cannula in the right femoral artery. Cerebrospinal fluid was obtained through a cisternal tap.

Two concentrations of ammonium acetate solution were used, 10.4 Gm. of ammonium acetate in 600 ml. of 5 per cent dextrose in water and 20.8 Gm. of ammonium acetate in 600 ml. of 5 per cent glucose in water.

Ammonia levels were determined by the method of Seligson and Hirahara.[9] Ammonia was liberated from a sample of whole blood in a diffusion vessel by contact with an alkaline buffered potassium bicarbonate mixture. The ammonia was collected by diffusion and measured colorimetrically. Values were reported in micrograms per milliliter of whole blood. All samples were analyzed in duplicate.

Samples for ammonia determinations were obtained simultaneously from the inflow and outflow vessel to an organ.

RESULTS

Liver Studies.—Control levels of ammonia in 15 animals were determined in the portal and hepatic veins before any ammonium acetate was infused (Fig. 2).

Supported in part by a grant from the U. S. Public Health Service, No. RG 4745-C.

Read at the Annual Meeting of the Society of University Surgeons, Boston, Mass., Feb. 13-15, 1958.

There was considerable variation in the amount of ammonia being presented to the liver via the portal vein in the different animals. Some of the animals had rather high portal levels, up to 3.5 mcg. per milliliter. The portal levels un-

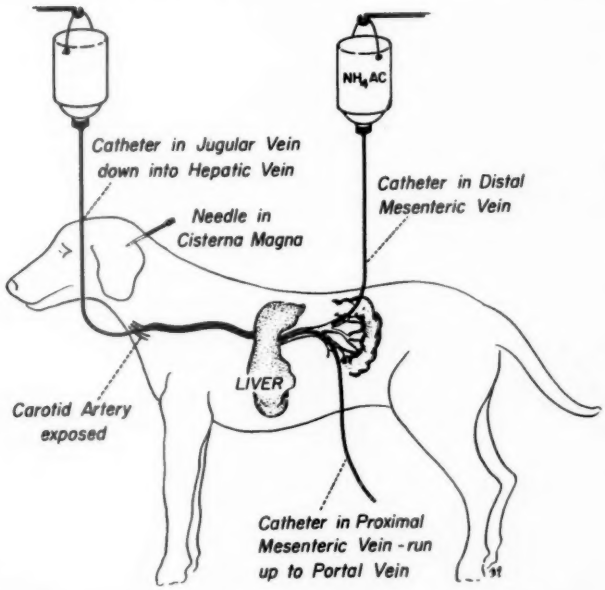

NH_4AC
Catheter in Jugular Vein down into Hepatic Vein
Needle in Cisterna Magna
Catheter in Distal Mesenteric Vein
LIVER
Carotid Artery exposed
Catheter in Proximal Mesenteric Vein - run up to Portal Vein

Fig. 1.—Diagram showing placement of catheters for simultaneous sampling for ammonia levels in various regions. A ureteral catheter was placed through the left jugular vein into the hepatic vein under fluoroscopic control. Through a right rectus incision a polyethylene catheter was threaded through a large mesenteric vein into the portal vein. A solution of 5 per cent dextrose in water was allowed to drip slowly in these catheters to maintain their patency. The right carotid artery and jugular vein and the left femoral artery and vein were exposed surgically to facilitate sampling. The right femoral artery was cannulated for direct measurement of blood pressure in some animals.

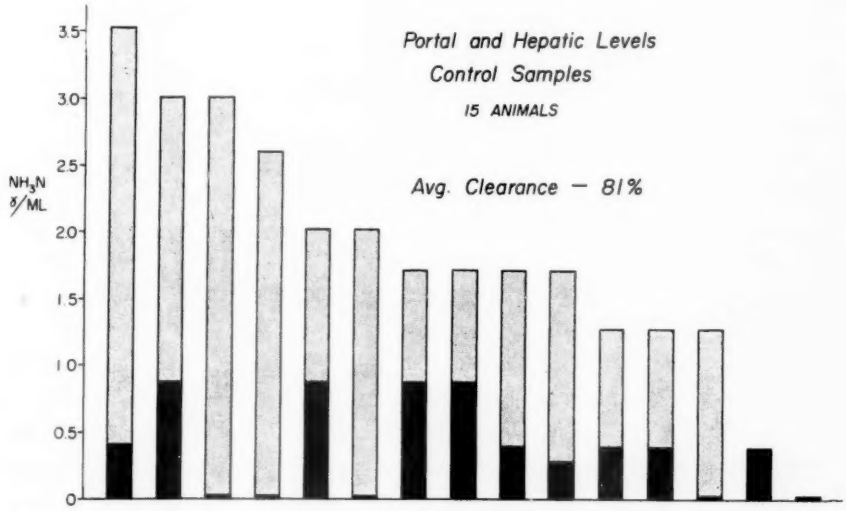


Fig. 2.—Portal and hepatic ammonia control levels in 15 animals. The portal level was plotted from the zero line up. Likewise, the hepatic level was plotted from the zero line up and is shown in black. The stippled area denotes difference between portal and hepatic levels.

doubtedly depended upon the time of eating and type of diet given the animal prior to the experiment. The average level in the portal vein was approximately 1.5 mcg. per milliliter.

Some of the animals cleared almost all of the ammonia going into the liver, as was evidenced by the very low levels in the hepatic veins. None of the animals showed any morphologic evidence of liver disease. When the levels in the portal and hepatic veins were compared, in all animals the average diminution in level was 81 per cent.

Immediately after control samples were obtained, infusion of the ammonium acetate solution in the mesenteric vein was started. During the infusion, one or two samples for ammonia determination were collected from the portal and hepatic veins. The infusion was then stopped, and after 10 minutes additional samples were collected. In most animals a second infusion was given and continued until the animal died of toxicity. The difference between the levels of ammonia in hepatic vein and portal vein was assumed to be the amount of ammonia "cleared" by the liver. The term "clearance" is used as an expression of the change in level of ammonia in the inflow and outflow vessel. Since organ blood flow was not determined, it is obvious that this is not actual clearance.

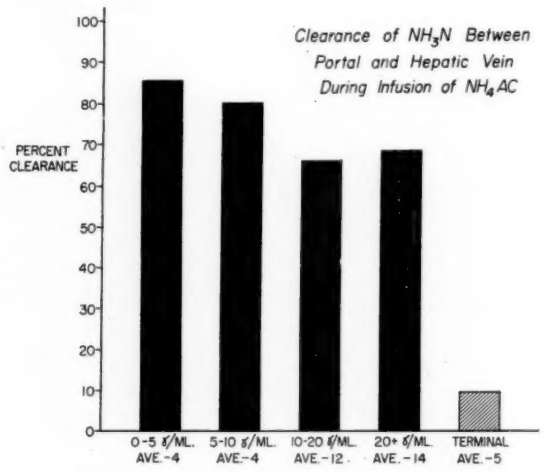


Fig. 3.—Graph showing percentage of clearance of ammonia (actually diminution in levels, not clearance) between portal and hepatic veins during infusion of ammonium acetate. At various levels of infusion the liver seemed to clear ammonia well. The levels in the portal vein were not any higher than the levels in the 10 to 20 or 20 plus mcg. per milliliter groups.

For practical purposes, however, it seems logical to speak of this change in levels as an amount of clearance of ammonia. The percentage of ammonia cleared under varying circumstances is shown graphically in Fig. 3. When the level of ammonia in the portal vein was between 0 and 5 mcg. per milliliter, the average amount of clearance in 4 animals was 85.2 per cent. When the level of ammonia was somewhat higher, between 5 and 10 mcg. per milliliter, the amount of clearance was 79.9 per cent. As larger quantities of ammonium acetate were presented to the liver, giving portal levels of 10 to 20 mcg. per milliliter and 20 or more mcg. per milliliter, the percentage of clearance was 66.4 and 69.4, respectively. The clearance for ammonia in these 4 groups was only slightly below the average control clearance value of 81 per cent. It is well known that concentrations of 20 or more micrograms per milliliter of ammonia is toxic for dogs.[3]

It appears from these data that the normal dog liver can clear tremendous quantities of ammonia. All dogs, however, showed a very low clearance of ammonia (9.7 per cent) in the samples taken just before death. This diminution in clearance, when the animal was in extremis, was not due to any abnormally large quantities being presented to the liver, since the portal levels were the same as in the 10 to 20 or 20 plus micrograms per milliliter group. It would appear that continued infusion of ammonium acetate was extremely toxic, and the liver failed to clear ammonia as a terminal event.

Liver Studies During Hemorrhagic Hypotension.—After control samples in the portal and hepatic veins were obtained, 4 animals were bled until the blood pressure fell to a systolic level of 60 to 70 mm. Hg. Ammonium acetate solution was then infused, with portal levels between 10 and 20 mcg. per milliliter. The average change in concentration of ammonia between the portal and hepatic veins was 61 per cent. This is slightly below the changes seen in the normal animals in the previous group. During hemorrhagic hypotension of short duration the liver was able to remove the ammonia presented to it very effectively until the animal was near death.

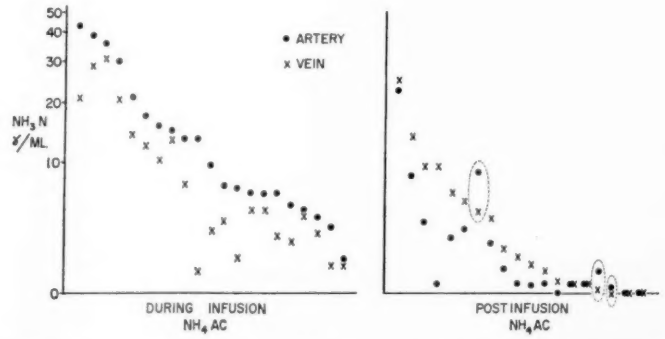
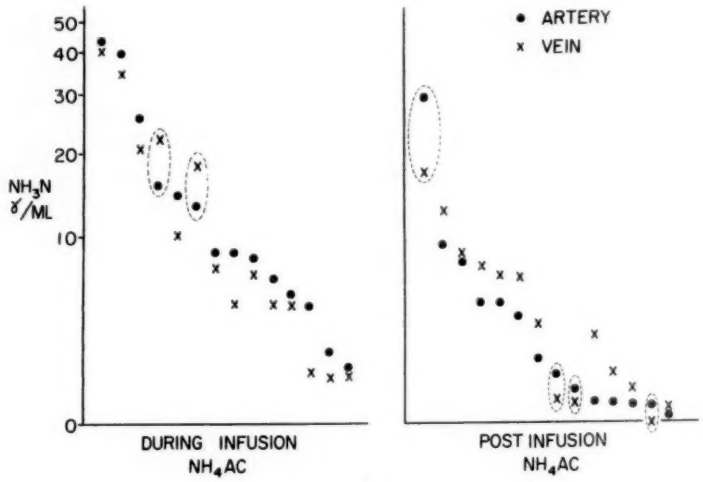


Fig. 4.—Levels of ammonia in random samples taken simultaneously from the femoral artery and vein in 10 animals. The level of the arterial sample was plotted as a dot. The level in the vein obtained simultaneously was plotted immediately above or below the dot in accordance with the level. The levels in the artery were always higher than the level taken simultaneously in the vein during infusion of ammonium acetate. After infusion the reverse was true in almost every instance. The 3 exceptions in which arterial was above venous levels during the postinfusion period are circled with a dotted line.

Muscle Studies.—Only a small amount of ammonia was present in the control samples from the femoral artery and femoral vein. Levels ranged from 0 to 0.86 mcg. per milliliter. There was little or no difference between the artery and vein in any of the control samples. Simultaneous samples were obtained from the artery and vein *during* and *after* infusion of varying amounts of ammonium acetate. The random determinations in 10 animals are plotted in Fig. 4. The level in the femoral artery was always higher than the simultaneous level in the femoral vein *during* infusion of the ammonium acetate. Levels taken 10 minutes or more after cessation of the infusion showed that in all but 3 instances the level in the femoral artery was equal or below the level in the vein. It would appear from these data that during the infusion of ammonia large quantities of the ammonium ion were presented to the muscle mass in the leg and that much of the ammonia did not return to the general circulation by way of the vein. As soon as the infusion was stopped, however,

the ammonia that was stored in the muscle mass was released back into the general circulation. This may offer an explanation for the differences in arterial and venous levels in patients with liver disease. If the amount of ammonia in the general circulation is increasing, the arterial level is higher, and, if the amount is decreasing, the venous level is higher.

Fig. 5.



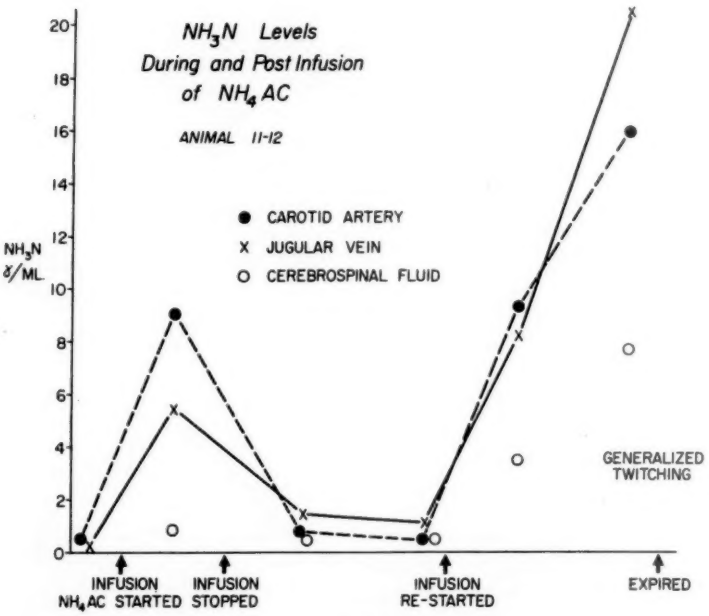


Fig. 6.

Fig. 5.—Random ammonia levels taken simultaneously from the carotid artery and jugular vein in 8 animals. The levels are plotted similarly to those in Fig. 4. The level in the carotid artery was higher than the level in the jugular vein during infusion, except in 2 instances. These are circled with a dotted line. After the infusion was stopped, almost all the levels in the vein were above those in the artery.

Fig. 6.—Graph showing ammonia levels in carotid artery, jugular vein, and cerebrospinal fluid in a typical experiment. With the infusion of ammonium acetate solution, the level in the artery rose rapidly as did the level in the vein. The level in the cerebrospinal fluid was elevated only slightly. After cessation of the infusion the level in artery, vein, and in the cerebrospinal fluid decreased to near normal levels. After the infusion was re-started, the ammonia level in the artery and vein rose, as did the level in the cerebrospinal fluid.

Brain Studies.—Simultaneous levels of ammonia in the carotid artery, jugular vein, and cerebrospinal fluid during and after infusion of ammonium acetate solution in 8 animals are shown in Table I. The differences between simultaneous determinations in the cerebral artery and jugular vein are plotted in Fig. 5. During infusion almost all of the carotid artery levels were higher than the jugular vein levels. Immediately after the infusion the levels in the vein were higher than those in the artery. These levels parallel those presented in the aforementioned determinations of femoral artery and vein samples.

TABLE I. SIMULTANEOUS LEVELS OF AMMONIA IN CAROTID ARTERY, JUGULAR VEIN, AND CEREBROSPINAL FLUID DURING AND AFTER INFUSION OF NH_4Ac SOLUTION

	LEVELS DURING INFUSION			LEVELS POSTINFUSION		
ANIMAL NUMBER	CAROTID ARTERY (MCG./ML.)	JUGULAR VEIN (MCG./ML.)	CEREBROSPINAL FLUID (MCG./ML.)	CAROTID ARTERY (MCG./ML.)	JUGULAR VEIN (MCG./ML.)	CEREBROSPINAL FLUID (MCG./ML.)
4-117	7.2	5.3	—	8.2	8.7	5.3
	5.8	5.3	4.3	0.6	0.3	0.3
	39.9	34.9	20.4			
11-12	9.2	5.3	0.8	0.3	1.2	0.3
	9.2	8.2	3.5	0.3	0.8	0.3
	15.9	21.8	7.7			
11-13	13.6	18.4	10.3	1.7	3.9	8.7*
	14.7	9.7	9.7	0.3	3.0	1.2*
11-14-1	8.7	7.2	0.8			
11-14-2	1.9	1.2	0.6	0.3	0.0	0.0
	2.5	1.0	0.3	1.0	0.3	0.8
	5.0	1.2	1.0	0.1	0.3	0.0
11-19	26.2	21.1	9.2	4.3	6.7	6.2*
11-22	66.4	68.5	14.7	9.2	13.0	21.8*
				5.3	6.7	13.0*
				5.3	8.2	6.7*
11-26	43.3	41.0	9.7			

*Cerebrospinal fluid levels that were above the simultaneous arterial levels.

Control determinations on the cerebrospinal fluid showed that there was little or no ammonia present normally. During infusion of the ammonium acetate solution cerebrospinal fluid levels were never found to be above the carotid artery or jugular vein levels. After infusion the cerebrospinal fluid level was above the arterial level in 6 instances and above the venous level in 3 (Table I). From these data it appears that after infusion ammonia was released from the cerebrospinal fluid rather slowly.

Carotid artery, jugular vein, and cerebrospinal fluid levels are shown in a typical animal in Fig. 6. As ammonia was infused, the level in the cerebrospinal fluid rose slightly but came down to a very low level after the infusion was stopped. Upon reinfusion of ammonium acetate the levels in the artery and vein were quite high, and the cerebrospinal fluid levels rose considerably. It would appear that the ammonium ion diffuses rather readily in and out of the cerebrospinal fluid. Further work, however, is necessary to determine more precisely the rate of diffusion.

COMMENT

In 1930, Bollman and Mann[3] found that elevations of blood ammonia in a range of 20 mcg. per milliliter were toxic for dogs. It would appear from

the data presented in this study that the liver removes large quantities of ammonia even at toxic levels until the animal is in a terminal, agonal state.

Nelson and Seligson[8] found that the liver reduced the concentration from the portal to the hepatic vein markedly in shock and that the concentration in the hepatic vein was elevated from normal. The amount of ammonia in the portal vein was increased during the shock state, but large quantities of the ammonium ion were not infused. In their study, as in our own, the liver of the shocked animal would remove ammonia but did not adequately reduce the hepatic vein level to a normal concentration.

Bessman[1] points out that in hepatic coma the arterial blood ammonia is normally slightly higher than the venous level, and, as the arterial level rises, the muscle takes out a fairly constant amount. He feels that muscle removes about 40 per cent of the arterial ammonia. During infusion of ammonia, however, the evidence presented in Fig. 4 shows that the level of ammonia in the artery is high and that after the infusion has stopped the level in the vein is above the arterial level. It would appear that the ammonia is taken up by the muscle, either intracellularly or in the interstitial space, and then much of it leaves the area as the level in the general circulation diminishes. Bessman[1] has also pointed out that as the blood ammonia rises in hepatic disease in patients, the brain as well as the muscle takes up ammonia and that the rate of uptake by the brain is about one-half that for muscle. The data in Fig. 5 give evidence that the brain takes up ammonia during infusion but also discharges it as the level in the general circulation falls. The cerebrospinal fluid level will rise as ammonia is being infused, but after the infusion has stopped the level will fall back to normal in a very short period in dogs.

From this study it appears that differences between arterial levels and venous levels of ammonia may be explained on the basis of an increasing or decreasing amount of ammonia passing through the liver into the general circulation.

Lawrence and co-workers[6] have infused ammonium acetate into eviscerectomized dogs. Their findings supported the thesis that peripheral tissues are a site of ammonia removal. They found that only 0.6 to 5 per cent of the injected ammonia could be accounted for in the extracellular fluid of all but one of the eviscerectomized dogs. In our studies, however, the fact that after infusion the venous levels were always higher than the arterial levels would point toward release after storage of ammonia in the brain and muscle. The changes in these acute experiments of high arterial levels during infusion and high venous levels after infusion show that the brain and muscle mass take up and release the ammonium ion rapidly. Whether this ammonia is held in the interstitial space or the intracellular space has not been answered by this study.

The rapid rise in venous ammonia levels after infusion makes it appear that the ammonium ion is a freely diffusible one. This fact makes it seem possible that the use of some ion-exchange resin in the gastrointestinal tract might reduce levels of ammonia in the blood during hepatic failure.

SUMMARY AND CONCLUSIONS

Ammonia levels were determined on samples of blood taken from various regions during and after the infusion of ammonium acetate solution into anesthetized dogs.

Control samples from the portal and hepatic veins taken before infusion of ammonium acetate solution showed an average diminution in levels of ammonia of 81 per cent by the liver. When large quantities of ammonia were infused with portal vein levels up to 20 or more micrograms per milliliter, the hepatic levels were markedly below portal levels except in samples taken just prior to death. In 4 animals with induced hemorrhagic hypotension the diminution in levels was almost as great as in the above group except immediately before death.

Samples taken simultaneously from the femoral artery and vein showed that arterial levels were higher during infusion and venous levels higher after infusion. Likewise, cerebral arterial levels were higher than jugular vein levels during infusion, and the venous levels were higher in the postinfusion period. This may offer an explanation for differences in arterial and venous levels in patients with liver disease. If the amount of ammonia in the general circulation is increasing, the arterial level is higher, and if the amount is decreasing, the venous level is higher.

Simultaneous carotid artery, jugular vein, and cerebrospinal fluid levels during and after infusion showed that ammonia passed readily in and out of the cerebrospinal fluid, but the changes in cerebrospinal fluid level were not as rapid as in the vascular tree.

We are pleased to acknowledge the technical assistance of Miss Mary Nelle Entrekin, M.T. (ASCP).

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Discussion

(Papers by Najarian, Harper, and McCorkle; Artz, Stanley, Eure, Langford, and Snavely)

DR. WILLIAM V. McDERMOTT, JR. (Boston, Mass.).—At present what we mean by the measurement of blood ammonia is certainly confused by technologic limitations.

The great trap in the field of amino acid therapy of ammonia intoxication is that the patients to whom we refer by the term "hepatic coma" comprise a very confused and amorphous group. Dr. Najarian has very rightly attempted to break these down into categories but, as I am sure he recognizes, this really is not possible because there is so much overlap in the effects of portal systemic collaterals bypassing the liver, the effects of parenchymal damage, and all the various combinations thereof.

As far as the treatment of ammonia intoxication per se, and this is by no means the only metabolic deficit associated with portal systemic collaterals and with liver disease, the effect of glutamic acid biochemically has become quite clear by now. In our experience, we have had a very consistent sudden fall in blood ammonia during the infusion of glutamic acid with a subsequent rise after the cessation of the infusion, if the source of the ammonia intoxication has not been controlled. Along with this, there is a consistent rise in glutamine and, again, a sudden fall as the ammonia rises, again indicating that this is a reversible reaction between glutamic acid and ammonia to form glutamate which then reverses.

There are several other interesting associated findings. One is that, in the presence of persistent ammonia intoxication, the citric acid level is often elevated and, following glutamic acid infusion, there is a rather steady fall in citrate levels, suggesting again that the interference of ammonia with the central nervous system metabolism is by direct entrance into the tricarboxylic acid cycle and the glutamic acid does have a remediable effect on this portion of the phenomenon.

Our experience with arginine has been quite varied. It is effective under some circumstances and the report given by Mr. Pearl would suggest perhaps some of the reasons for the inconsistency in the treatment of ammonia intoxication with arginine. It certainly does have a role, if we understand the situation under which it should be used, and perhaps measurements of citrulline and arginine will allow us to determine when this can be effective and when it actually may be dangerous.

Arginine glutamate was a compound which we approached with considerable interest, but I am very puzzled by it. We have not seen any consistent effects as we have with glutamic acid or even in some circumstances with arginine. I am not sure whether arginine glutamate acts as a peptid in the body or whether it functions as a salt. It is a very peculiar compound.

DR. BEN EISEMAN (Denver, Colo.).—We have utilized arginine in the treatment of over 40 cases of clinical hepatic coma and have found it useful but by no means as effective as have Drs. Najarian, Harper, and McCorkle. As with glutamate, it will, in our experience, lower circulating blood ammonia concentration, but all too frequently it will only temporarily halt the progressive and unrelenting course of the disease.

My feeling is that it is time to focus our attention not on the substrate discrepancies of liver disease but on defects in enzymes that most probably underlie these problems.

DR. WALTER LAWRENCE (New York, N. Y.).—In regard to Dr. Artz's arterial-venous level with ammonia infusion, I have a slide that has some bearing on this. We took all of the abdominal viscera out of a group of dogs and then injected ammonium acetate intravenously by just one injection and followed the arterial ammonia following this. You can see from this slide that the arterial ammonia level rose immediately but then dropped almost to the control values. Repeating this gave us a similar result.

We tried to get both arterial and venous levels during this sort of experiment, but we could not find any real arterial-venous difference which was any greater than the error

of the method. We used a method similar to that described. We felt that this did demonstrate some significant degree of peripheral detoxification of ammonia over and above that which would ordinarily be done by the liver. The liver, of course, had been removed from these animals.

We have been mainly interested in the treatment of patients with glutamate. By now, we have some 150 patients upon whom we have tried sodium glutamate, and our clinical impression of the large group was that about two-thirds of the patients who did not continue to bleed were significantly improved, both so far as blood ammonia and clinical status were concerned. Dr. VanAmmey in our laboratory has reviewed more critically a group which were better documented. There were approximately 30 patients in this group, and these confirmed our impression that about two-thirds of the cirrhotic patients did have significant drops in blood ammonia and improvement in clinical status. If bleeding continued or patients developed pneumonia or if they were of the chronic hepatic insufficiency group discussed by Dr. Najarian, our results were not as good.

We have tried arginine glutamate because it seemed like a solution to the problem. A few trials of arginine that we had in our laboratory were not very effective, but we did not give an adequate trial. Of 5 patients given arginine glutamate, 3 patients came out of coma with lowered blood ammonia.

I would only inject one note of caution about interpretation of the clinical effect of any of these drugs, and that is this: In all the patients we have treated enemas have been given to remove this source of ammonia. They have received other supportive measures, such as electrolyte abnormalities. They also received glucose infusions. It is hard to honestly say that we are sure that this two-thirds of the patients improved by glutamate actually can be attributed entirely to glutamate.

DR. WILLIAM SHOEMAKER (Boston, Mass.).—The gradient changes that Dr. Artz and his collaborators have measured, combined with flow measurements, would lend themselves to clearance techniques very easily.

We have done some preliminary work with measurement of hepatic clearance of ammonia in association with Dr. Rodke in the biochemical department. We have measured this with hemorrhage and after food, a large protein meal, and have found some fairly remarkable changes. We have found the method that Dr. Rodke has worked out for the measurement of ammonia is vastly superior to the usual methods which are largely modifications of Conway's technique.

DR. HENRY THOMAS RANDALL (New York, N. Y.).—These papers have added some more information to our knowledge of this disease and perhaps some more confusion to our general thinking. This is a process of evolution.

I can recall some 10 years ago being in the same position in regard to the role of potassium. I am not sure that we know entirely what its role is today.

These papers certainly show excellent progress, particularly in the comment with regard clinically to the level of ammonia concentration in the cerebrospinal fluid and the subsequent confirmation of this by the animal studies of Dr. Artz. This gives an answer, at least an approximation, to why so many of these patients under treatment have a lag period before they respond to treatment. This slow rate of build-up of ammonia in the cerebrospinal fluid and its relatively slow rate of drop give us a perfectly logical explanation of this phenomenon which has made us wonder previously whether our treatment was off the beam as far as it might have been.

We are making progress. I suspect that in another year or 2, we shall have further information from many of these fine workers which will tell us enough of this story that we can use it even more effectively.

DR. NAJARIAN.—As Dr. Randall indicated, our understanding of the relation between ammonia intoxication and the clinical states designated as hepatic encephalopathies has been improved by the finding of increased concentrations of ammonia in the cerebro-

spinal fluid after the blood ammonia concentrations have decreased in patients who are in a state of hepatic coma. When unconscious patients are admitted to the hospital, determination of the cerebrospinal fluid ammonia may help to identify the cause of coma.

We have used arginine as an adjunct to the elimination of the production of ammonia in the gastrointestinal tract in our treatment of patients with hepatic coma. It is used only when patients are stuporous or in coma from ammonia intoxication. In these cases it is important to rapidly reduce increased concentrations of ammonia because irreversible brain damage might occur to patients in prolonged hepatic coma. In many of our cases of hepatic coma blood ammonia concentrations have been effectively decreased with arginine therapy. The recent use of arginine glutamate for this purpose has appeared to give us an even more rapidly effective method of controlling ammonia intoxication.

DR. ARTZ (Closing).—I appreciated Dr. McDermott's comment particularly when he said that we are not sure what we are measuring. In the patient we are still not certain what we are measuring by the ammonia technique. In the ammonia that was infused, we were probably measuring the ammonium ion.

I thought that maybe Dr. Lawrence would comment a little further about his work and conclusions from the experiments in the eviscerectomized animals. It was his feeling that very little of the ammonia was in the extracellular space.

I am not certain how to interpret our data of the high venous levels after the ammonia infusion had ceased, but some place in the muscle mass and in the brain is a level of ammonia. Whether this is in the interstitial space and comes out so rapidly or not, I am not sure. Like the other individuals, clinically we feel that the cerebrospinal fluid level is probably our best indication of the depth of the disease in patients with hepatic insufficiencies.

WOUND HEALING: A STUDY OF THE RESPONSE OF INJURED TISSUES TO THE COENZYME ADENOSINE 5-MONOPHOSPHATE

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(From the Department of Surgery, University of Maryland School of Medicine)

SURGERY as a science and surgical technique as an art of that science is founded upon the premise that man possesses a native tendency to repair wounds. Field asepsis, gentleness with tissues, hemostasis, suture composition, and incision variations have developed as the surgeon has attempted to assist that tendency through his understanding of the repair process. Until recently gross observations of rate of wound contracture and histopathology furnished most of the known information concerning repair. Within the last 5 years biochemical techniques have furnished data describing normal wound healing patterns in terms of the sequential formation of mucopolysaccharides and collagen. Dunphy and Udupa[8] demonstrated that formation of mucopolysaccharides preceded collagen formation in the early stages of healing and fell rapidly as collagen accumulated. They suggested 2 stages of wound healing: an initial or productive phase "in which the building blocks of repair are formed" and a collagen phase, continuing until contracture and healing are complete. When similar wound studies were carried out on protein-depleted animals, methionine was found to restore toward normal levels mucopolysaccharide accumulation and collagen formation otherwise retarded by protein starvation.[14] Williamson[15] showed a direct correlation between increase in tensile strength and retention of sulfur in granulation tissue. Morgan and Morton[12] demonstrated dependence of fibroblasts on sulfur amino acids by pointing out that the intact animal probably converts methionine to cystine; this conversion is accompanied by fibroblastic growth and function.

These apparently isolated events have in common a dependence upon connective tissue cellular biokinetics, for each substance investigated represents a product of cellular synthesis. Altering cellular activity with exogenous substrates may offer new clues in further elucidation and elaboration of repair. The present study is concerned with the effect on cellular dynamics of one substrate, coenzyme adenosine 5-monophosphate, as represented by biochemical alterations in wound healing after mechanical, metabolic, and steroid stress.

MATERIALS AND METHODS

Three hundred young albino rats each weighing between 150 and 200 Gm. were subjected to control or experimental factors for 5 days. On the evening of the fifth day under light ether anesthesia and following depilation, a 2 cm. wound was made on the back of

Read at the Annual Meeting of the Society of University Surgeons, Boston, Mass., Feb. 13-15, 1958.

each animal. For uniformity a cork punch was employed to mark the area of section. Aseptic technique was used and the dissection was carried down to the deep fascia. No dressings were applied.

On postwounding days 1, 3, 7, 12, and 20, specimens were obtained by sacrificing sample animals in each group. Prior to sacrifice all animals were weighed. Sections of normal tissue at a distance, wound periphery, and wound center were obtained for histologic examination and for determinations of collagen, glucosamine, tyrosine, and total protein.

Control Evaluation.—Thirty animals were placed on normal diets* before and after wounding. No additional factors were added to this regimen.

Mechanical Stress Group.—Thirty animals were placed on normal diets; pre- and postwounding each animal received 20 mg. of adenosine 5-monophosphate (A5MP) daily.†

Metabolic Stress Group.—One hundred and twenty animals were placed on Williamson's[15] zero protein diet before wounding. Postwounding this group was divided into 4 series: (a) 30 animals were continued on zero protein diets; (b) 30 animals were continued on zero protein diets and pre- and postwounding these animals received 20 mg. of A5MP daily; (c) 30 animals were continued on zero protein diets and postwounding only, these animals received 20 mg. of A5MP daily; and (d) 30 animals were returned to normal diets.

Steroid Stress Group.—One hundred and twenty animals were maintained on normal diets throughout the experimental period. For 5 days prewounding each animal received 6 mg. cortisone acetate daily. Postwounding this group was also divided into 4 series: (a) 30 animals were continued on 6 mg. of cortisone acetate daily; (b) 30 animals were continued on 6 mg. cortisone acetate daily and pre- and postwounding each animal received 20 mg. of A5MP daily; (c) 30 animals were continued on 6 mg. cortisone acetate daily and postwounding each animal received 20 mg. of A5MP daily; and (d) in 30 animals cortisone acetate was withheld postwounding and each animal received 20 mg. of A5MP daily in the postinjury period.

Physical Analysis of Tissue.—Immediately after sacrifice serosanguineous exudates, if present, were removed from the wound. Color, density, and depth of granulations were appraised. Measurements of size of wound were recorded, and a color photograph was taken of each animal.

Chemical Analysis of Wound Tissue.—Collagen determinations were indirectly computed from hydrolysis according to the method of Neuman and Logan.[13] Since this procedure requires extraction with acetone and ether, results are expressed as milligrams per 100 Gm. of dry weight after extraction.

Glucosamine values were computed from the scheme of Elson and Morgan[9] as modified by Blix[2] and Boas.[4] In our work fresh tissue was weighed in covered microbalances immediately after section; results are expressed as milligrams per 100 Gm. of fresh tissue.

Histopathologic Analysis of Tissue.—Representative sections of tissue from animals of each group were fixed and stained by the following methods: hematoxylin and eosin, Gomori's reticulin stain, van Gieson's collagen stain, and periodic acid-Schiff for demonstration of mucopolysaccharides. Our sections included normal tissue on opposite borders of each wound.

RESULTS

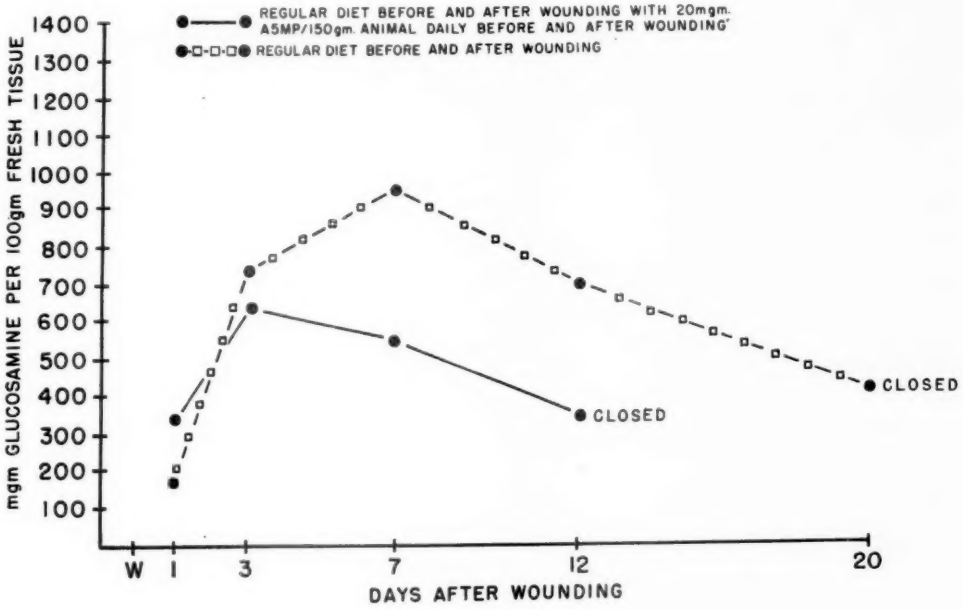
The results are shown in Figs. 1 through 6. The course of healing and the results of the chemical studies in each group are presented.

*Rat Friskie Chow.

†The adenosine 5-monophosphate utilized in this work was supplied by Ames Company, Inc., Elkhart, Ind., and known as My-B-Den.

Mechanical Stress.—There is a definite difference noted between the control animals and those in which A5MP has been supplemented. For 3 days postwounding, control animals lost 8 to 10 Gm. of body weight but recovered this loss by the fifteenth to the twentieth day. Those animals receiving A5MP lost less than 4 Gm.

Fig. 1.



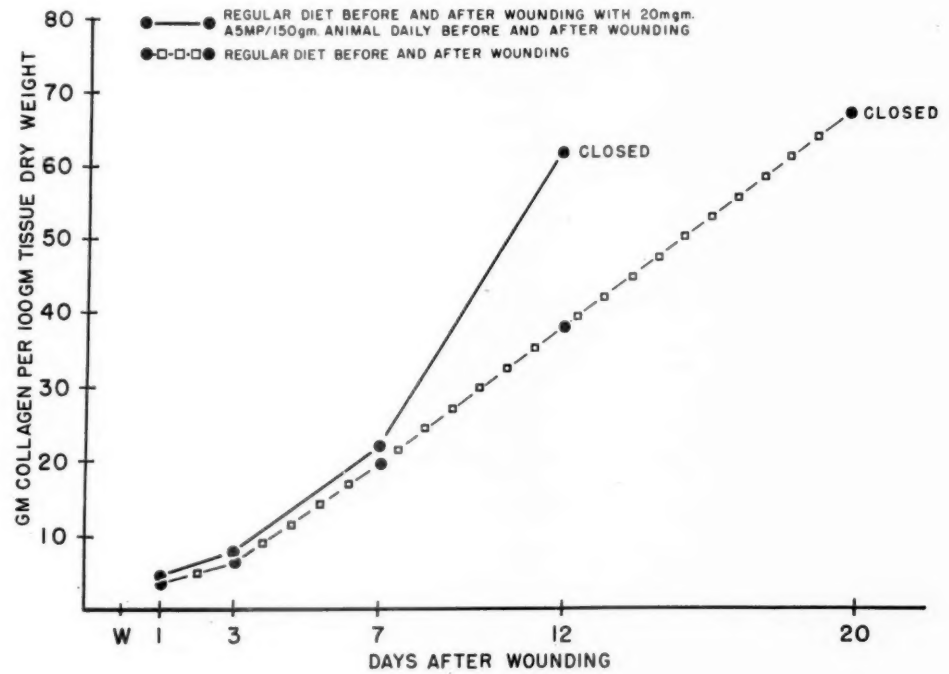
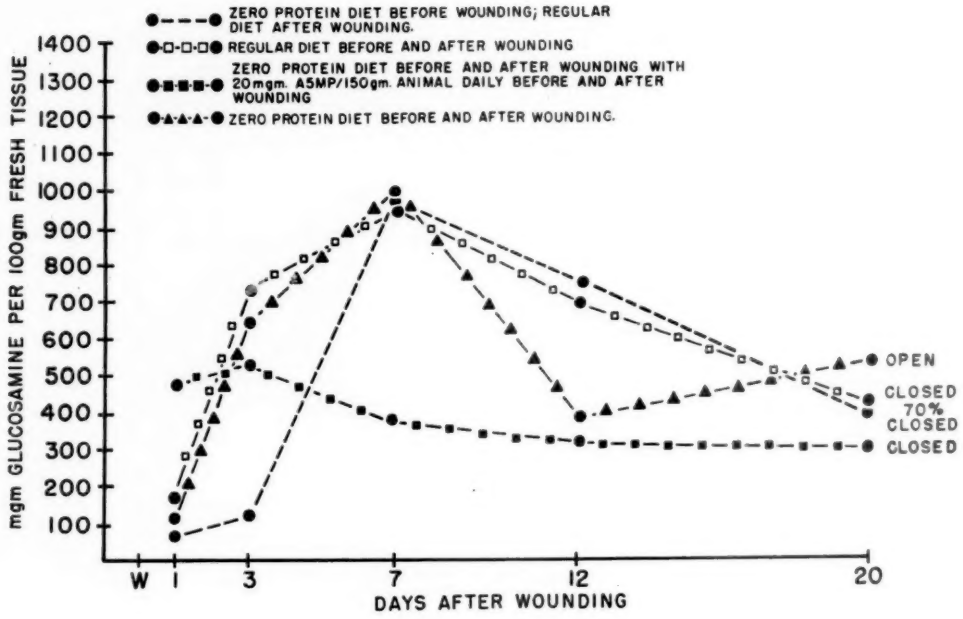


Fig. 2.

It is of interest that chemically the glucosamine concentrations in the A5MP animals reached peak levels with subsequent decline before those of control animals (Fig. 1). Concomitantly collagen values in the A5MP animals rose more rapidly than did those in the control group (Fig. 2). This is confirmed grossly when it is noted that the wounds in the A5MP animals closed

Fig. 3.



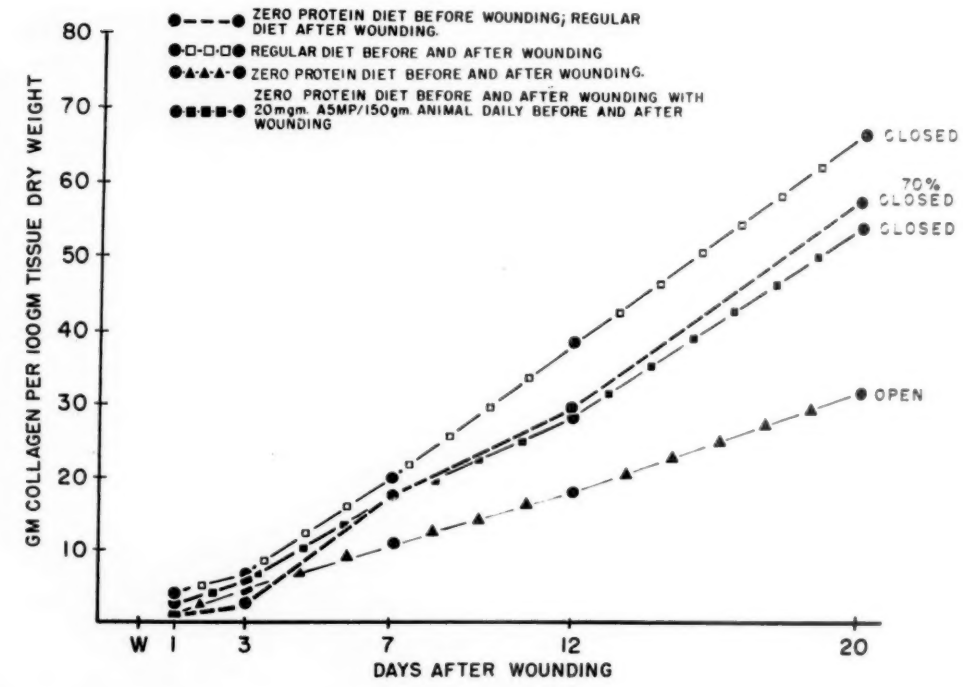
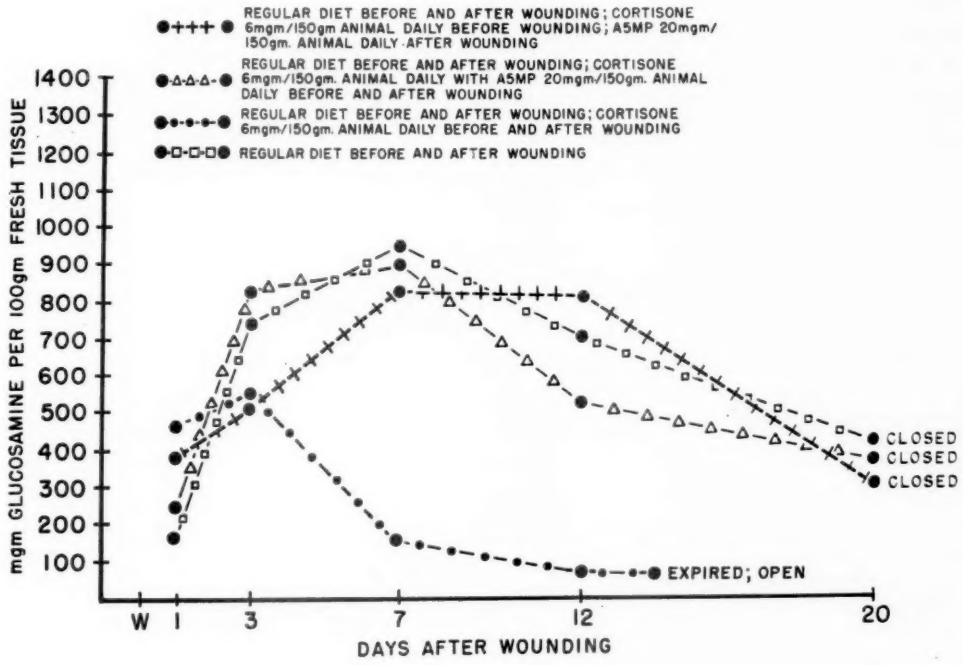


Fig. 4.

by the twelfth day while 30 days elapsed before complete healing in the control animal.

Metabolic Stress.—There is a striking difference between protein-depleted animals and those in which A5MP has been supplemented. Non-A5MP-treated animals sustained prolonged weight losses of 45 to 50 per cent by the twentieth

Fig. 5.



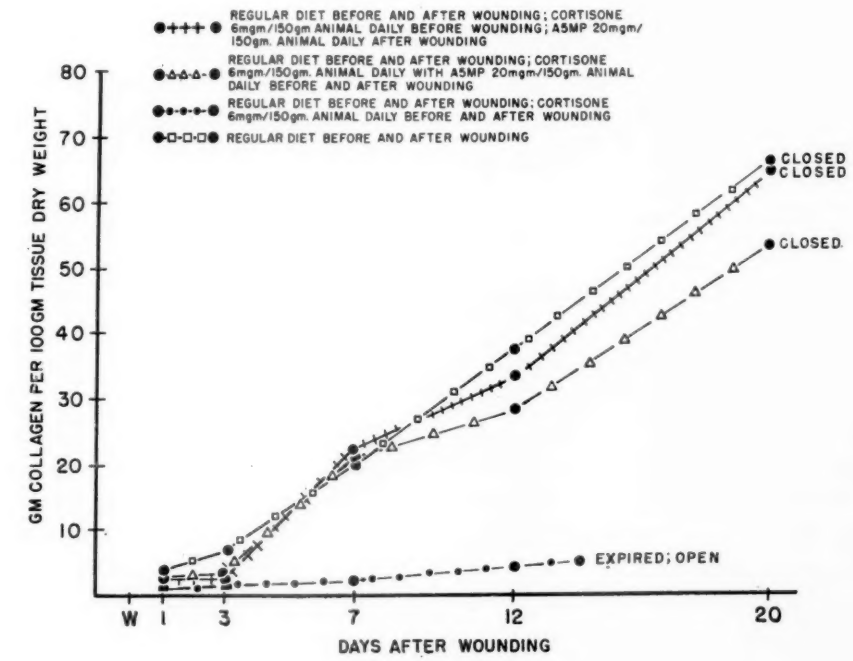


Fig. 6.

day. Those receiving A5MP declined less markedly; losses did not exceed 30 per cent of the prewounding weight. Chemical determinations confirmed clinical appearances. In the animals which received no A5MP glucosamine values rose slowly, reached a peak on the seventh day, and gradually declined. Collagen content of these wounds rose slowly after the seventh day but remained low. Wounds were still open on the twentieth day. Animals which received A5MP pre- and postwounding showed glucosamine and collagen values to parallel those of normal animals. The wounds closed completely by the twentieth day. In the animals treated with A5MP postwounding only, chemical values show less collagen formation and only 70 per cent closure of wounds by the twentieth day postinjury.

The group which was returned to regular diet postwounding but received no A5MP regained a 20 per cent weight loss, but their wounds showed a decrease in collagen formation and only 70 per cent wound closure by the twentieth day (Figs. 3 and 4).

Steroid Stress.—This group was apparently the most severely stressed in our study. Non-A5MP-treated animals expired by postwounding day 15. The animals sustained a 10 to 15 per cent loss in weight. Several developed oral and rectal bleeding. Chemically glucosamine values rose initially but declined rapidly after the third day. Collagen remained low, never approaching normal values. In the A5MP-supplemented animals chemical analysis revealed a pattern similar to that of protein-depleted A5MP-treated animals—early glucosamine rise and fall with gradual rise in collagen between days 3 and 7 and a more sustained rise between days 7 and 20. It is of especial interest to note the group in which cortisone was stopped postwounding and A5MP was administered. Glucosamine values rose to the highest levels by the third day, leveled through the twelfth day and fell to near normal levels by the twentieth day. Collagen formation was delayed 24 to 30 hours but rapid increase occurred after the twelfth day with wound closure by the twentieth day (Figs. 5 and 6).

Correlation of chemical results with histologic sections, the spatial effect demonstrated by comparison of normal and peripheral tissue with those of the wound, and tyrosin evaluations as protein determinants are the subjects of separate reports.

DISCUSSION

Collagen synthesis is stimulated by adenosine 5-monophosphate (A5MP) (Figs. 2, 4, and 6). Regardless of stress, mechanical alone or with protein starvation or excessive cortisone dosage, relative and absolute collagen content was increased in wounds of those animals whose recovery was improved by addition of A5MP pre- and/or postwounding. This collagen effect is especially demonstrated by those animals in group c of the cortisone stress series; withholding cortisone postwounding and substituting A5MP in that interval resulted in rapid deposition of collagen and wound closure (Fig. 6). Synthesis of general body protein is apparently nurtured by A5MP during the collagen

phase of healing (Fig. 4). Not only did collagen accumulate in wounds of protein-starved animals with A5MP added to the regimen but increased overall body weight resulted after the third postwounding day.

Particularly striking is the closure effect demonstrated by all A5MP-treated animals (Figs. 2, 4, and 6); it will be noted that wounds in this series had closed completely by the twentieth postwounding day. Yet wounds of prewound protein-starved animals failed to close even though restored to normal diets postwounding (Fig. 4). This observation may be explained by increase in tensile strength and implicate A5MP in sulfur metabolism, most likely in cystine synthesis. Transmethylation is mediated through activated S-adenosyl methionine,[11] the S-N derivative of A5MP.

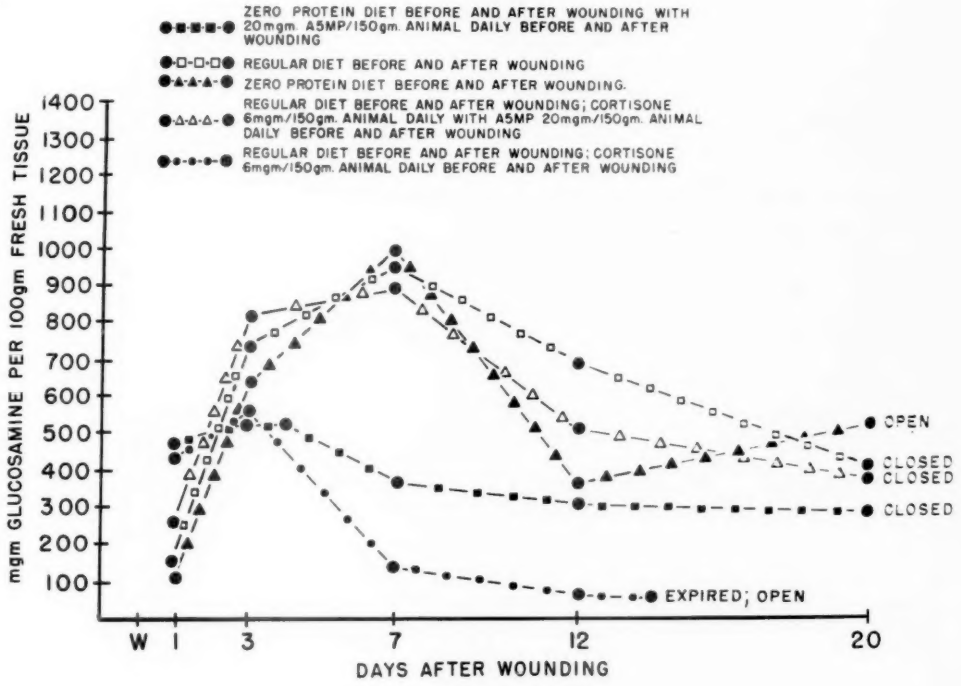


Fig. 7.

Hexosamine utilization is presumably augmented by A5MP (Figs. 1, 3, 5, and 7), for initially higher levels fall more rapidly when the substrate is added to animal regimens. Hexosamine content reached a peak just before the period of rapid collagen synthesis; since fibroblastic activity has been demonstrated to require sulfur amino acids,[12] the interdependence of amino sugars and sulfur amino acids does not seem random or unrelated. Possibly energy for fibroblastic activity may be supplied primarily via the formation of aminohexoses. This may be likened to series specificity and conservation of energy by the organism, for less free energy is relinquished in this pathway of carbohydrate metabolism.

A5MP is a cellular substrate composed of adenine, ribose, and monophosphoric acid. It has been isolated in uncombined form in extracts of rat

and rabbit brain and muscle and in rabbit kidney, and its free form has been inferred or predicted for several other organ systems.[7] The physiologic importance of this substrate derives from it participation in reaction sequences of energy formation, storage, and utilization, and in synthesis. Glycolysis and aerobic oxidation provide most of available cellular energy; most if not all free energy so liberated (except that dissipated as heat) is coupled to energy-rich pyrophosphate bonds of adenosine triphosphate (ATP).[10] The free energy change associated with hydrolysis or synthesis of each of the two pyrophosphate bonds of ATP amounts under physiologic condition to about 14 Kcal.[5] That A5MP is active in "charging" ATP has been shown by Albaum[1] who has demonstrated prompt blood level elevations after intramuscular administration of A5MP. Exogenous ATP may be active as an energy source; however, greater activity can be achieved by generating ATP continuously by enzyme means, in which adenosine diphosphate (ADP) and inorganic phosphate are mobilized in the reaction sequences of the tricarboxylic acid cycle.[3] A5MP concentrations may be critical in maintaining ADP levels.

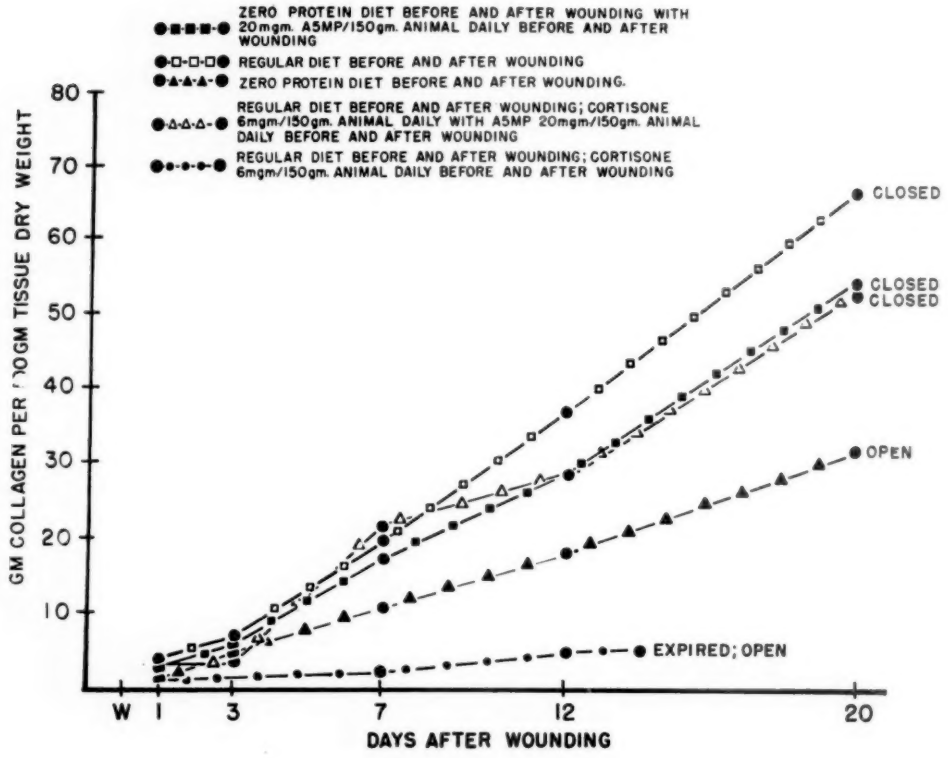


Fig. 8.

In a corollary pathway of carbohydrate metabolism, the conversion of galactose to glucose is mediated through uridyl diphosphonucleotides,[6] whose formation in mitochondria is a reaction sequence dependent upon A5MP. Uridyl coenzymes are related chemically to coenzymes I, II, and A which activate formation of several structures in glycolytic and oxidative breakdown of carbohydrate.

Effective over-all substrate activity is indicated by a comparison of the collagen effect in protein-depleted and cortisone-stressed animals (Fig. 8). Recovery values almost coincide in the collagen phase.

Such demonstrated substrate effectiveness indicates that A5MP influences two major areas of cellular activity: energy metabolism and synthesis. As the latter is dependent upon the former, the over-all effect may be that of promoting a more rapid return to aerobic processes and the decrease of lag interval in wound healing.

SUMMARY

1. Wound healing retardation is diminished in rats previously stressed by mechanical wounding and protein starvation or steroid stimulation if A5MP is added to the regimen.

2. Collagen production and hexosamine utilization are promoted in this process.

3. A5MP apparently mediates these processes by its effect on cellular energy reactions and in synthesis.

4. These studies suggest that A5MP may be effective in decreasing the lag interval of wound healing.

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DISCUSSION

DR. EDWARD L. HOWES (New York, N. Y.).—I do not doubt the efficacy of what you have done in the chemistry discussed. I wish to make only one point: that the so-called University Surgeons must stop talking about wound healing. Wound healing is two processes: epithelization and the formation of granulations or fibroplasia. There is also the regeneration of blood vessels. Then, when one considers an open wound like this, contraction, in which the surrounding tissues simply pull things together, is added. All

the systems of chemistry are so different that they cannot be considered effectively together. The system of chemistry for epithelization requires the nucleic acid synthesis. Amino acid synthesis takes place in the formation of collagen fibers. The presence of certain specific amino acids, such as hydroxyproline, is necessary for the synthesis of collagen fibers. To jump from nucleic acid synthesis to amino acids and then add wound contracture is giving too much too fast to reassemble without difficulty. Have you really found an anticortisone? This is extremely important, because all wounds heal under the influence of cortisone and, if we can use an anticortisone, this is an extremely important contribution in spite of my criticism.

DR. J. ENGLEBERT DUNPHY (Boston, Mass.).—I would endorse the statement of Dr. Howes that we must all learn to consider the components of repair and not wound healing. This is a study of certain components of granulation tissue during the processes of contraction and it raises the question as to whether the agent used has its effect in the wound or as a systemic phenomenon. The animals which were given massive doses of cortisone and then pretreated were much healthier animals by the time their wounds were examined than they would have been otherwise.

There is some systemic component to this as well as perhaps a local one in the wound. I would ask the authors whether or not they made any glucosamine determinations of serum because we have become very suspicious of the meaning of hexosamine in granulation tissue and suspect that most of it comes from the lack of proteins in serum and that its apparent fall during repair is related to the production of new tissue, and, if it is delayed, it is a relative fall as new tissue is formed. It is delayed in repair and protein depletion and cortisone because of the fall, because the production of new tissue is delayed.

DR. LEVENSON.—I would like to ask the authors if they made an attempt to control the amount of ingestion and realized the effect of the diet and the quantities actually eaten?

DR. REYNOLDS (Closing).—To answer Dr. Levenson's question concerning the amount of food ingested by our zero protein animals, we did control that amount by limiting them to approximately 7 Gm. per day. We did not limit the amount of food consumption on the animals which were on a regular diet.

We do not feel that this work demonstrates an anticortisone effect. Other investigators have demonstrated that in the production of a granuloma, the administration of A5MP produces apparently an ACTH lag effect. We hope to show later that A5MP perhaps augments the activity of cells directly under stress, that is, around the periphery of the wound and in the center of the granuloma or granulation tissue per se, rather than demonstrating an anticortisone effect.

We did not have any blood levels as far as glucosamine values are concerned, but our subsequent work will try to tie that in.

SERUM LACTIC DEHYDROGENASE IN THE DIAGNOSIS OF THE ACUTE SURGICAL ABDOMEN

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THE enzymatic activities of blood serum have been found useful in the diagnosis of a number of surgical and nonsurgical diseases. The activities in serum of various intracellular enzymes such as glutamic oxaloacetic transaminase (SGOT), have been shown to rise following surgical operations, fractures, hepatitis, and myocardial infarction.[3] Serum lactic dehydrogenase (LDH) has been observed to rise following myocardial infarction.[5]

This investigation tests the value of serum LDH activity in the preoperative diagnosis of necrotic intestine secondary to volvulus or mesenteric vascular occlusion. The enzyme LDH was chosen because of its relatively high concentration in intestine and intestinal mucosa.[2]

MATERIALS AND METHODS

The method of measurement used is essentially that of Wacker, Ulmer, and Vallee.[5] Under the experimental limits described, the changes in optical density are directly proportional to the time of incubation (Fig. 1) and to the amount of enzyme added to the serum. Highly active sera may show decreasing rates after the first few minutes. In such cases the rate is calculated from the early linear part of the reaction, or the serum is diluted.

TABLE I

	STOCK	FOR EACH DETERMINATION	REACTION MIXTURES
DPN	36 mg./ml. distilled water 50 mM/L.	0.5 ml.	8 mM/L.
d-l sodium lactate	0.17 M (3.25 ml. of 60 per cent solution/100 ml.)	1.0 ml.	0.06 M/L.
Sodium pyrophosphate buffer pH 8.8	0.1 M (44.6 Gm./L.) sodium pyrophosphate 500 ml. + 10 ml. 1 N HCl	1.3 ml.	0.043 M/L. pH 8.3
Serum	----------	0.1-0.2 3 ml.	

Reagents.—The stock reagents and reaction mixture are shown in Table I. In actual practice these may be mixed and measured into the 8 ml. test tubes in which the test is run. These tubes can be stored frozen in the emergency room. Either 0.1 or 0.2 ml. of unhemolyzed serum is placed in the tube after the reagents have been warmed to 33° C. in the water bath.

Supported in part by grants from the U. S. Public Health Service—RG-4192 (C3)—and the Harry Freund Memorial Foundation.

Read at the Annual Meeting of the Society of University Surgeons, Boston, Mass., Feb. 13-15, 1958.

The LDH activity of most sera is stable at room temperature for several days; however, that of highly active sera frequently declines more rapidly. Determinations done in the emergency room may be checked in the morning.

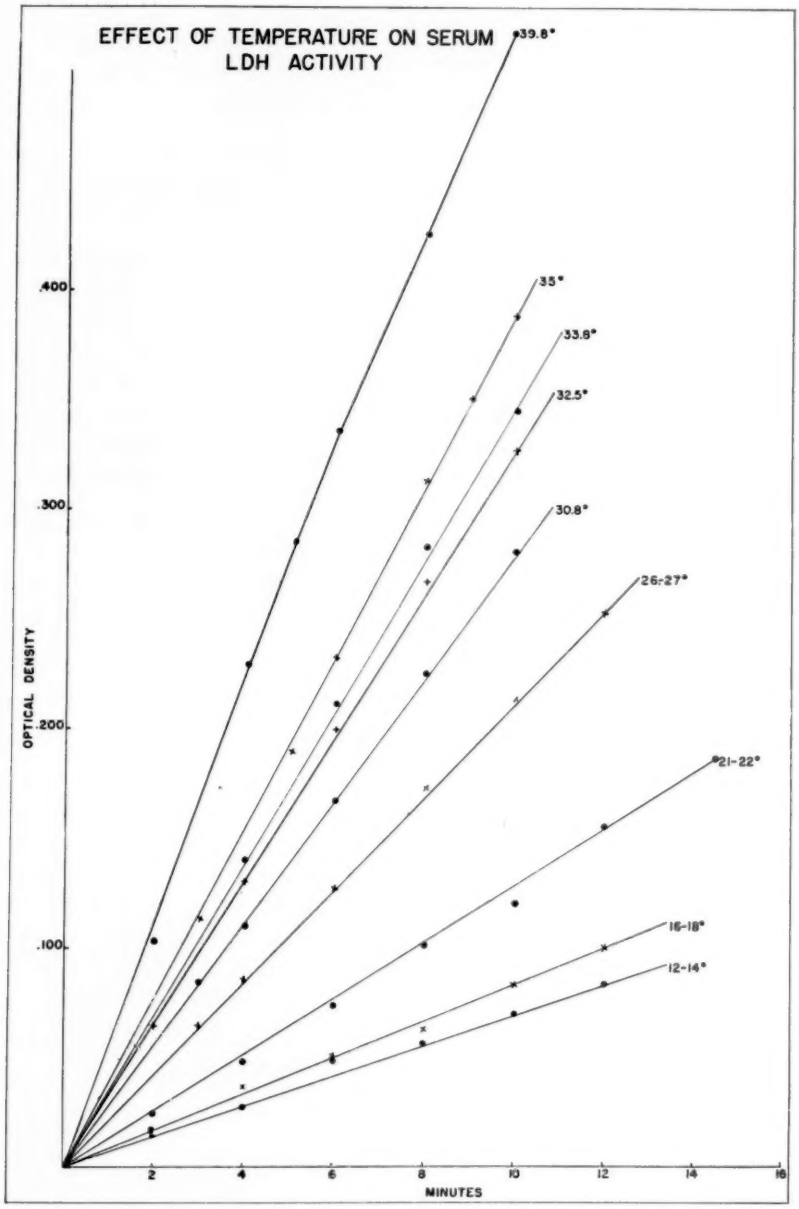


Fig. 1.—Effect of temperature on serum LDH activity.

Instrumentation.—A great deal of the ease of determination is due to the use of a simplified photoelectric colorimeter, the Coenzometer,* which operates at 340 to 350 mμ. After serum is added to the reagents, the mixture is transferred at once to the Coenzometer for an initial setting. Subsequent readings are taken every 2 minutes thereafter for 10

*Developed by Vallee, B. L., Margoshes, M., and Thier, R. E., at the Biophysics Research Laboratory, Peter Bent Brigham Hospital, Boston, Mass. Supplied by MacAlister-Bicknell Co., 243 Broadway, Cambridge, Mass.

minutes. Between readings the tube is kept in a water bath at 33° to 34° C., corresponding to the internal temperature of the instrument. The direct meter readings are converted to optical densities from a conversion table, and the LDH units are calculated as change in optical density per minute per milliliter of serum times 1,000. Line voltage regulation will improve the stability of the instrument in some locations.

Sources of Error.—Sensitivity and reproducibility depend upon accurate temperature control during incubation. The marked acceleration of the reaction at higher temperatures is shown in Fig. 1. Hemolyzed blood samples show large increases in activity, as LDH is released from the destroyed blood cells. While it is possible to arrive at an opinion with a hemolyzed serum after photoelectric measurement of the hemoglobin content, it is best to avoid hemolysis of the samples.

RESULTS

Normal Observations.—With the method described, one can obtain, with 5 consecutive determinations on the same sample, a reproducibility of ± 5 per cent. In a group of 22 normal individuals the average value for serum LDH activity was 111 units with a standard deviation of ± 35. The range was 26 to 212. These values are plotted in the first column of Fig. 3. The normal value, deviation, and range are comparable to those reported by other investigators when correction is made for differences in methods.[1, 5, 6] Day-to-day serum LDH activity in normal individuals is illustrated in Fig. 2.

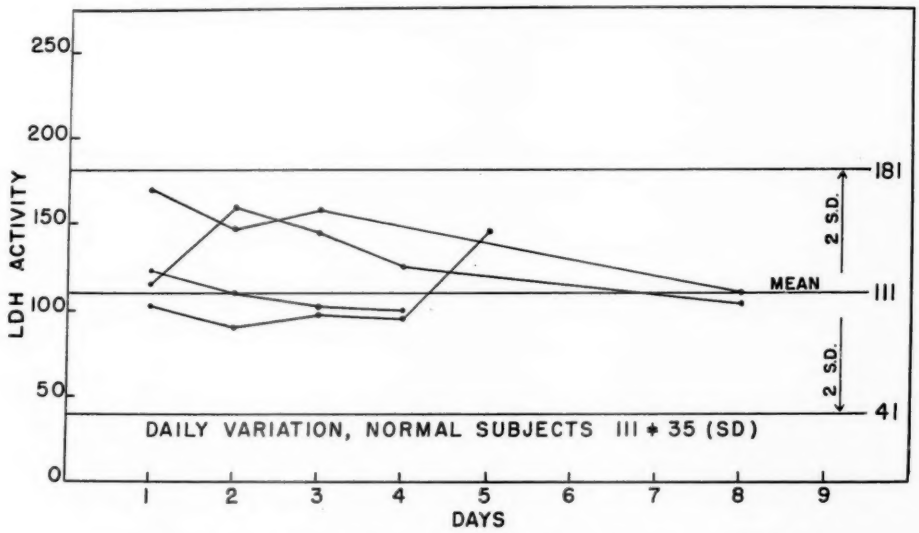
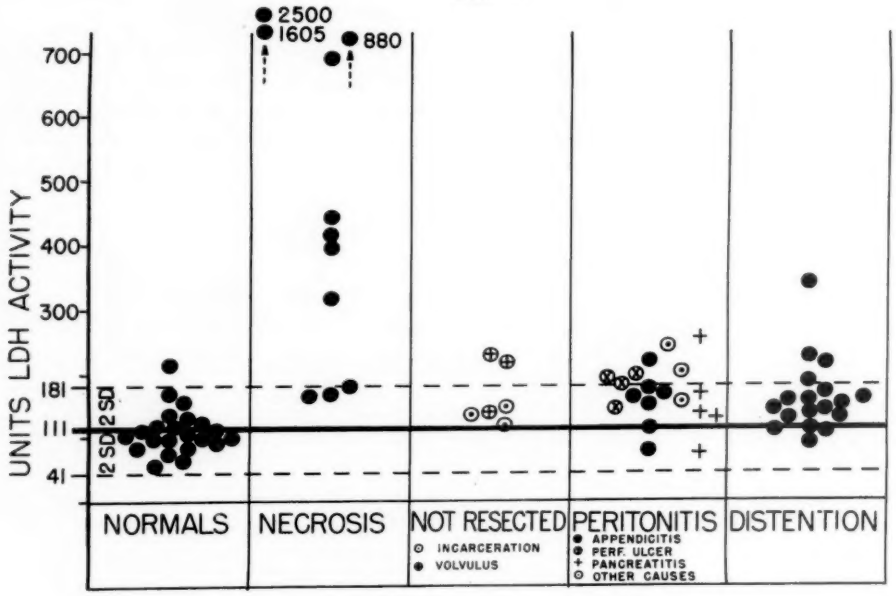


Fig. 2.—Daily variation of LDH activity in normal subjects.

Intestinal Obstruction With Intestinal Necrosis.—The preoperative serum LDH activity in 11 patients with necrosis of the intestine is shown in the second column of Fig. 3. All patients had resections of the bowel, and most had striking elevations of the LDH activity. One of the cases with normal serum LDH had obstruction of the mesenteric veins, because the intestine had passed through a small (2 to 3 cm.) opening. There was also a distal 180 degree volvulus of the trapped segment. Another case with a normal LDH was a strangulated femoral hernia requiring resection of only 3 inches of bowel.

The highest LDH activity was observed in a patient who had superior mesenteric artery embolism. Another patient had a coincident myocardial infarct with mural thrombosis.

Fig. 3.



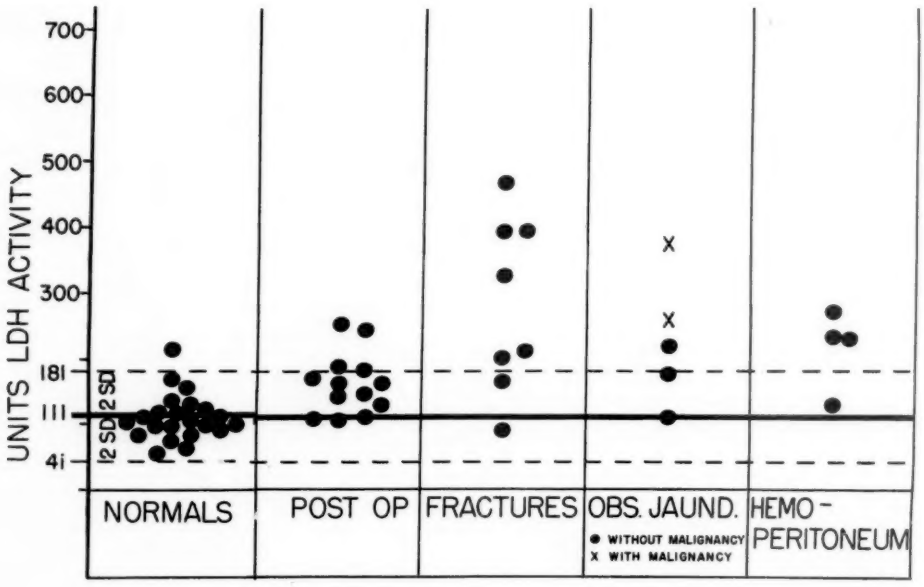


Fig. 4.

Fig. 3.—Normal subjects and patients with obstruction or signs of obstructions. Normals are mixed ages, male and female, and taken without regard to fasting. All other samples are preoperative values. The second column records those cases in which resection of intestine was necessary, regardless of other signs. The third column records those cases of volvulus or incarceration in which resection was not necessary. Cases of column 4 are localized or generalized peritonitis without gross distention. Column 5 shows serum LDH activities of patients with gross abdominal distention regardless of peritonitis or other mechanism of distention.

Fig. 4.—Normal subjects and the effects of operation, severe trauma, obstructive jaundice, and hemoperitoneum. The postoperative values are taken 24 hours after major procedures such as pneumectomy, gastrectomy, craniotomy, sympathectomy, or colectomy. The fractures are all of one or both femurs. The cases of hemoperitoneum are traumatic injury or ruptured ectopic pregnancy, all confirmed by laparotomy.

In several instances there was an opportunity to correlate repeated measurements of LDH activity with the clinical course of patients with infarcted bowel. Subsequent case reports illustrate the relationship.

The time that elevations of serum LDH activity occur and the degree of elevation are variable. The duration of the process and the amount of bowel infarcted are important, as is the venous drainage from the affected segment, a circumstance illustrated particularly by the case of volvulus and incarceration of the ileum already cited. The amount and degree of absorption of bloody peritoneal fluid are probably another factor since hemolysis of blood liberates large amounts of LDH.

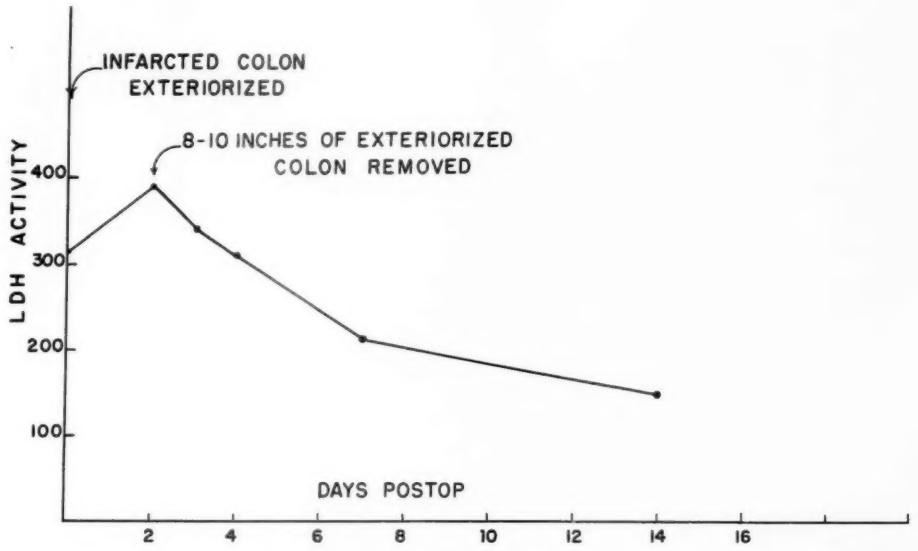


Fig. 5.—Case 1, infarction of right colon.

*Case 1:** R. M., a 12-year-old boy (Fig. 5), had an appendectomy. The acutely inflamed appendix was very long and lay retrocolic behind the mesentery of the hepatic flexure. This was edematous and friable. During the removal of the appendix, a hole was made in the mesentery. On the fifth postoperative day, the WBC was 28,000 and the serum LDH was 312. The following day laparotomy revealed a mass in the right lower quadrant; most of the right colon was purple and friable and was therefore exteriorized.

Two days later the serum LDH had risen to 395. At this time the necrotic exteriorized bowel was removed, following which LDH activity declined to normal levels.

Case 2:† J. K., a 63-year-old man (Fig. 6), was admitted for hemorrhoids. Four days later he developed acute volvulus of the sigmoid that was not reducible by sigmoidoscopy. At laparotomy several feet of necrotic sigmoid were resected. The superior hemorrhoidal vessels were thrombosed. The distal segment left after resection was of questionable viability. Serum LDH at the time was 694.

By the fourth postoperative day necrosis of the bowel at the distal suprapubic colostomy was evident. The serum LDH, after an intermediate decline, rose to 400. The following extraordinary peak (1,165) probably corresponded to additional infarction as confirmed at the time of elective resection and low anastomosis of the colon.

*We are grateful to Dr. Wm. Sinkler, M.D., Medical Director of the Homer G. Phillips Hospital, for permission to include these cases.

†We are grateful to Dr. Cesar Gomez of the St. Louis University Service, U. S. Veterans Administration Hospital, for permission to include this case.

In this case smaller amounts of infarcted tissue produced rises in LDH activity comparable to that from several feet of bowel in the same individual.

From the data available, serum LDH activity of greater than 300 units in the presence of intestinal obstruction should suggest the possibility of intestinal necrosis. Rises of this magnitude have not been observed in other abdominal diseases, as shown in the remainder of Figs. 3 and 4. Several cases of volvulus of the small bowel and incarcerated hernia, shown in column 3 of Fig. 3, showed no elevations and did not require resection.

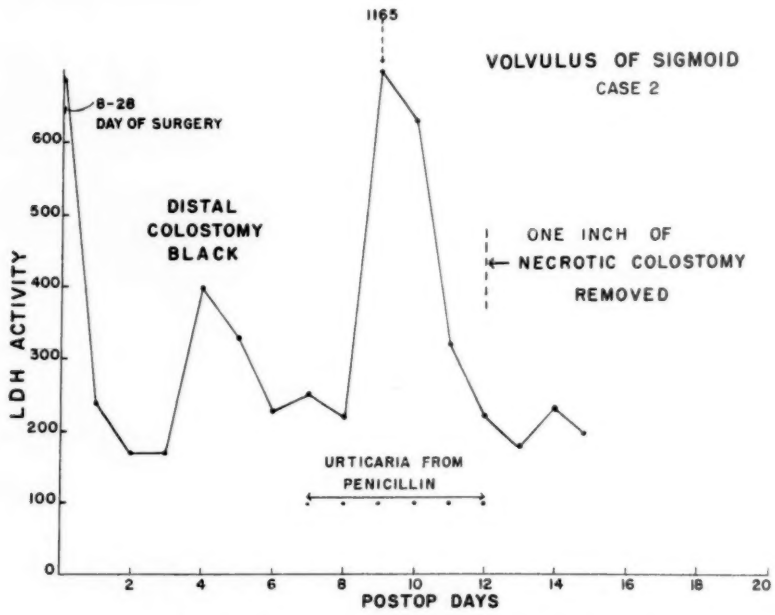


Fig. 6.—Case 2, infarcted volvulus of sigmoid.

Intestinal Distention.—Distention, with or without peritonitis, does not result in elevations of serum LDH. The 18 cases which are exhibited in column 5 of Fig. 3 include mechanical small bowel obstruction due to adhesions, obstruction from carcinoma of the colon, and postoperative paralytic ileus. Many of these patients had massive distention, but the majority of cases were of lesser degree. There is no correlation between the degree and duration of distention and LDH levels.

Peritonitis.—The cases of peritonitis which were not obstructed are depicted in column 4 of Fig. 3. Further data are needed for acute cholecystitis.

Necrosis of Other Tissues.—In 2 patients with incarcerated umbilical hernias containing infarcted adipose tissue, the serum LDH was normal.

In 5 cases of pancreatitis no striking elevations were observed, even though simultaneous serum amylase was over 1,000 units in 4 cases.

Case 3: J. E. (Fig. 7) was admitted for a recurrent attack of acute pancreatitis. The day following admission the serum amylase was 1,280 units, and the serum LDH was 256.

Laparotomy 10 days after admission revealed an indurated pancreas and recent fat necrosis. No stones were found in the gall bladder or common duct. Duodenotomy and sphincterotomy revealed a common channel. Following the fall in amylase postoperatively, a single moderate rise in serum LDH is observed. The cause of this rise in unknown but is probably not due simply to operative trauma.

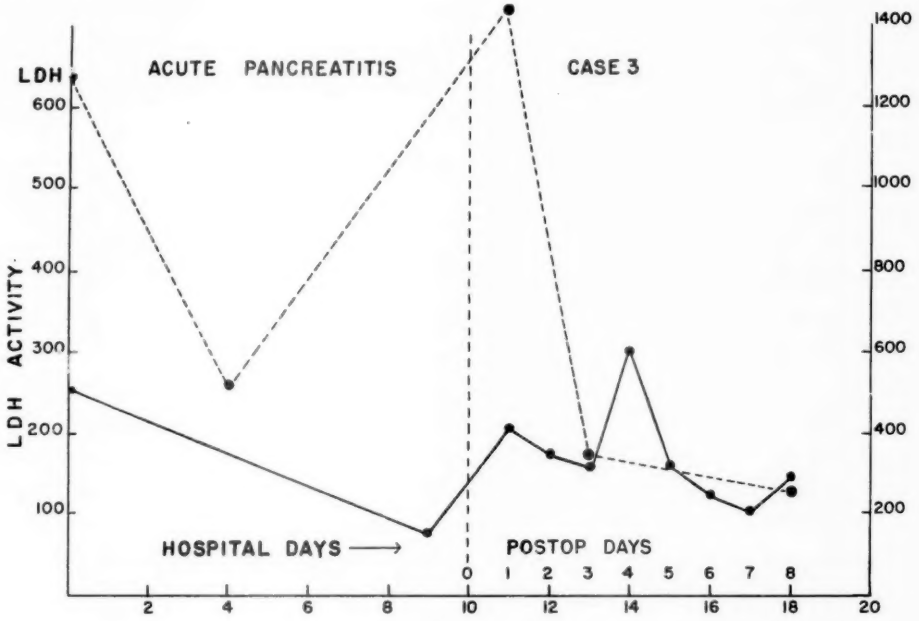


Fig. 7.—Case 3, acute pancreatitis.

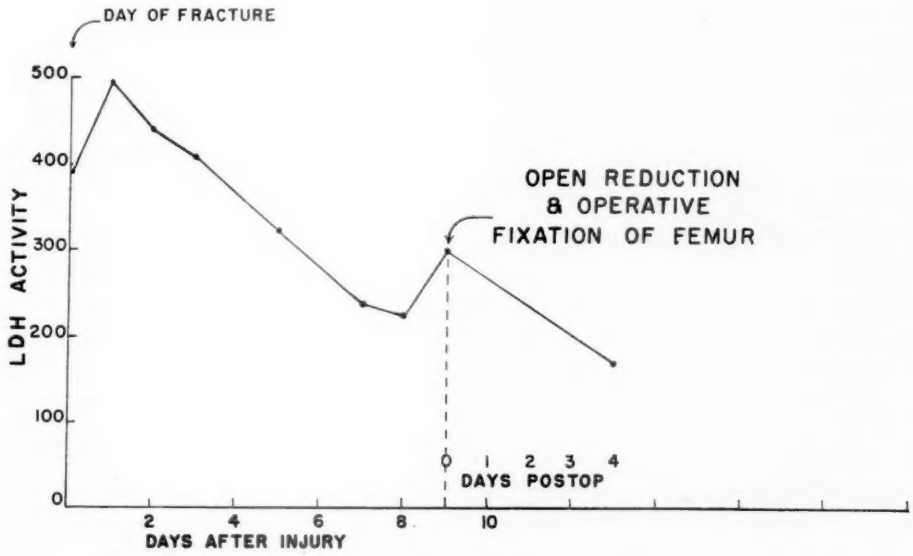


Fig. 8.—Case 4, fracture of femur.

Following surgical operations of considerable magnitude moderate elevations of serum LDH may be observed, as shown in column 2 of Fig. 4. These elevations may be more striking in the first few hours following surgical trauma;

however, after 24 hours, a level of over 300 should suggest the possibility of complications such as myocardial or intestinal infarct.

Trauma.—The trauma of major fractures causes considerable elevations of serum LDH activity (column 3, Fig. 4) which may persist for several days. Fig. 8 demonstrates this, and also reveals a secondary rise following internal fixation of the fracture. Absorption of LDH from the RBC of the fracture hematoma, as well as muscle damage, may play a role in elevation. Additional data on the effect of hemoperitoneum are necessary.

DISCUSSION

The presumed origin of increased serum lactic dehydrogenase activity in these cases is from intracellular enzymes released following cellular damage. Other possibilities such as activation or release of inhibition of serum LDH have been proposed. Whatever the mechanism, the association with dead tissue is apparently constant and, in addition to the experience recorded here, has been recorded by others in association with myocardial, renal, or pulmonary infarction and hepatitis.[1, 3, 5] The only exceptions to this are the high levels associated with widespread malignant disease and with thorazine hepatitis.[1, 2] Here necrosis cannot be excluded but is difficult to estimate. From our data thus far it does not appear that false positive examinations would be troublesome or confusing. If the serum LDH is over 300 in a patient with intestinal obstruction, necrosis of intestine is almost certain unless there is some other tissue coincidentally infarcted or damaged. High serum LDH is a more specific index of infarction and may be more sensitive than fever or leukocytosis.

The explanation of false negative examinations is difficult, and their occurrence is troublesome. In most clinical cases the time of onset or tissue death is difficult to estimate. From our experience with 3 cases of mesenteric artery occlusion, 6 to 12 hours from the onset of infarction seem a sufficient time to effect a rise, but more experience with this point is necessary, and a single normal serum LDH will not exclude infarction.

The rate of enzyme release and the possible routes of release and absorption are unknown. Regarding excretion and destruction, a single injection of purified enzyme causes a sharp rise in serum activity that disappears within 2 to 6 hours.[6] It would appear that the time of sampling is therefore of considerable importance and that repeated examinations will reduce the incidence of false negative results.

The accumulation of further data on LDH in acute pancreatitis may be of value in the separation of those cases of intestinal strangulation that are confused with pancreatitis because of high serum amylase.

Other enzymes doubtless enter the serum in intestinal and other tissue infarcts. It is conceivable that a number of such methods could be developed to reflect changes accompanying a particular disease or disease of a particular organ. Practically, the use of LDH has the advantages of economy, rapidity, and technical simplicity.

CONCLUSIONS

1. Elevation of serum lactic dehydrogenase (LDH) of more than 3 times normal has been observed in 8 of 11 cases of intestinal infarction.

2. Mechanical small bowel obstruction without necrosis, intestinal distention from other causes and of varying degree, generalized or localized peritonitis, and surgical operations of large magnitude have not caused such striking rises.

3. A rapid, reliable, simple procedure for the determination of serum LDH in the emergency room is described.

4. The diagnostic value and limitations of the determination are discussed. Serum LDH activity appears to be more specific for intestinal necrosis than other laboratory criteria; however, it will rise after infarction of other tissues including myocardium, lung, and kidney, and this must be considered in differential diagnosis.

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DISCUSSION

DR. F. B. HERSHEY.—We were interested in this subject particularly as a help in the early diagnosis of mesenteric thrombosis. Some of these cases have insidious onset and come to surgery after several days on the medical wards and we find that we must perform massive resections.

How useful it is going to be in early diagnosis we cannot say. In all of Dr. Calman's cases except this last one with the massive volvulus, the diagnosis was already made clinically. It was of interest, however, that the serum LDH was elevated, although in many of these patients the white blood cell count, temperature, pulse were normal and some of the other laboratory signs looked for in diagnosis of intestinal strangulation were all negative. Therefore, we did not need the LDH to make the diagnosis in the patients he describes.

We must go to the dog laboratory to learn more about the time course, how early the LDH goes up. We will also need experimental work to find out how much intestine must be strangulated. We have tried to work out another enzyme method before we do that. In this slide, you will see that the LDH on the left is abundant in practically all the tissues that we have measured. In the next 3 columns are enzymes which are relatively specific for certain tissues. The one of particular interest in intestinal necrosis is the third column. These figures show that it is much higher in activity in the bowel than it is elsewhere. One of the problems that troubled Dr. Calman was the differential diagnosis of intestinal necrosis compared with myocardial infarction, for example. Perhaps if we can measure this enzyme in the serum, and I think that we can now, we will be able to have a more specific method than the LDH would offer. The diamino oxidase for kidney tissue is rich in kidney and

sparse elsewhere, and glycolic acid oxidase is relatively specific for liver tissue. In all this work on enzyme diagnosis of various disease states, we could pay more attention to enzymes that may be more specific for the particular tissue that we are trying to diagnose.

One other caution—as in the case of the amylase, you are still going to have to be a surgeon. There is no laboratory test that we can ever give you that will avoid that.

DR. ELLIS.—We have gone to the dog laboratory in an attempt to see how early we could see changes in certain enzymes that could be measured. We were interested primarily in transaminase values. This seemed to be a logical study, since this has been shown to be elevated in the presence of necrotic or dead tissue.

To date, in the literature, serum transamination has been of little help. However, in the laboratory, we have found that arterial or venous occlusion of the superior mesenteric artery or even many of its small branches has resulted in marked rises in the transaminase values in the peritoneal fluid.

Although we have not tested it clinically, it looks as if it might be of value to do peritoneal taps and determine transaminase values and, we suspect, intestinal faction.

PHYSICAL AND BIOLOGIC FACTORS IN IMPEDANCE PLETHYSMOGRAPHY

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(From the Departments of Experimental Surgery and Medical Electronics, Albany Medical College of Union University)

THE impedance plethysmograph is an instrument which will measure and continuously record small resistance changes in a segment of biologic tissue. Changes in the volume of an electrical conductor will produce changes in total resistance so that this instrument functions as a volume measuring device. Cyclic volume, and therefore resistance, changes occur in an extremity as a consequence of the pulsatile nature of arterial inflow and the steady rate of venous outflow. In order to measure blood flow by means of the impedance plethysmograph, it is therefore necessary to establish a quantitative relationship between resistance change and volume change and also to translate the measured cyclic volume change into blood flow. This report will describe an impedance plethysmograph which provides accurate volume measurement and also a method for deriving limb blood flow from the volume data obtained.

The quantitative measurement of blood flow is essential for the interpretation of the results of medical and surgical therapy for peripheral vascular disease. Indirect estimations based upon skin temperature, sweating, and subjective improvement of symptoms do not necessarily correlate with changes in total limb blood flow.[4] The impedance plethysmograph offers the practical advantages of ease of clinical application, high sensitivity, and frequency response, and therefore the possibility of calculating blood flow on a pulse-to-pulse basis. The present study was undertaken with standard measuring circuits so that the biologic factors which determine the total impedance could be separately measured over a wide frequency range. The relation between electrical resistance and volume was then measured in mechanical models, in the isolated hindlimb of the dog, and in the intact human forearm by comparison with an air plethysmograph.

The original work of Nyboer,[5] as well as later circuits designed by DuBois and Nims[2] and van den Berg,[7] assumed that an extremity could be considered as a series of parallel resistors in which one, the volume of blood in the limb, was the only variable. Under these circumstances, the change in total resistance due to change in the volume of blood in the limb could be easily calculated. In practice, however, biologic tissues have an impedance which results from electrical properties other than simple resistivity. These properties can be grouped

This work was supported by a grant from the United States Air Force.

Read at the Annual Meeting of the Society of University Surgeons, Boston, Mass., Feb. 13-15, 1958.

under the heading of capacitive reactance and result principally from the dielectric effect of the skin and other low conductive tissues. The capacitive effect, if uncompensated, will introduce an error of unpredictable magnitude.

Schwan[6] deduced from theoretical and experimental data that body tissues may show high capacitance effects, and therefore the over-all impedance of the limb, which is the vector sum of resistive and capacitive impedance, could not be directly related to limb volume. With the technique to be described, it is possible to separately determine both capacitive and resistive impedance and also to balance out the capacitive effect. Under these conditions, the relation between relative volume change and relative resistance change should be $\frac{\Delta r}{R} = \frac{\Delta v}{V}$ where R and V are the total resistance and volume, respectively, and Δr and Δv are small changes due to the pulsatile change in blood volume.

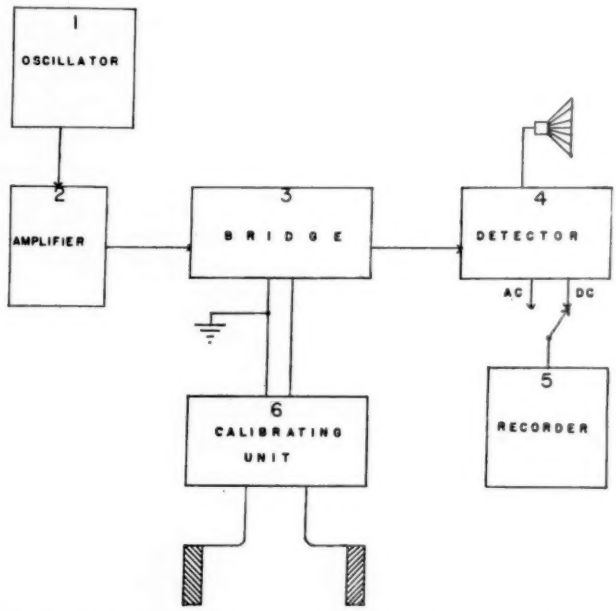

Fig. 1.—Schematic diagram of impedance plethysmograph. *1*, 200 KC crystal-controlled oscillator. *2*, Amplifier and cathode follower. *3*, Radio frequency bridge. *4*, Modified radio receiver. *5*, Direct-writing recorder. *6*, Decade calibrating resistors 0.1 and 1.0 ohm.

INSTRUMENTATION

The instrumentation used in this study has consisted mainly of the basic General Radio type 916AL radio frequency bridge, with associated generator and detector accessories.

Initially, a General Radio type 1330A variable frequency oscillator was used as the generator. The detector consisted of a Hallicrafters S X 28A receiver and an RME type LF 90 low-frequency converter. This system provided the flexibility for a determination of a suitable operating frequency and sensitivity. By means of the RF bridge, reactive impedances associated with the unknown resistance could be quantitatively determined. Nonreactive resistance decades are used for calibration.

Experimentation on intact limbs indicated that once the electrode application had stabilized (5 to 10 minutes) the reactive component of the unknown impedance appeared as a small capacitance but did not vary. Any tendency toward a pulsatile change of capacitance

was investigated by slowly swinging the incremental reactance dial of the bridge through the balance point while observing the change of resistance riding slightly up on slope toward the resistance null point. No detectable pulsatile wave-form variation, other than amplitude, was noticed. This effect was determined over a frequency range up to one megacycle. It was then concluded that in the case of pulsatile volumetric measurements, the capacitive reactance could be balanced out leaving a corrected small resistance change for numerical determinations. This same procedure was carried out following venous outflow occlusion and also indicated a negligible effect. The instrumentation has been modified to have more radio frequency voltage available at the source to improve the over-all sensitivity of the device to small changes due to pulsatile changes in the extremity. A 200 kilocycle, 80 volt source was built using a crystal oscillator, amplifer, and cathode follower. A new receiver was built around a government surplus low-frequency command unit, SC1317A. The choice of 200 kilocycles for operating frequency was quite arbitrary, being more or less determined by the relative expense of different units. Simple voltage-regulated plate power supplies are used. The radio frequency bridge is still the basic measuring unit. It has been our feeling that popular circuitry should be used as far as possible for these basic studies. A portable electric plethysmograph has been built employing the items mentioned. This instrument is illustrated in Fig. 1. Assuming that the capacitive reactance of the unknown impedance is a constant, an electric plethysmograph using either voltage drop or a balance bridge circuit should be a valid instrument. Over-all electric circuit stability and ease of application and operation are the most important features.

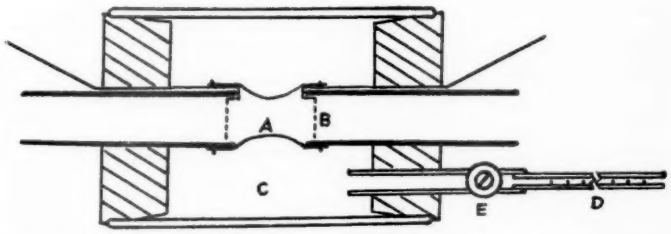

Fig. 2.—Mechanical model for measuring small volume changes. *A*, Thin rubber tube. *B*, Electrodes. *C*, Water jacket. *D*, Calibrated burette. *E*, S opcock.

METHODS

The relation between impedance and total volume for irregular, geometric objects has been studied. In order to carry out this measurement, it was decided to use a mechanical model with a precisely known initial volume which could be varied by specific increments. The construction of this model is shown in Fig. 2. The volume of the water jacket (*C*) can be changed by applying pressure to the calibrated burette (*D*) and thus altering the volume of the test chamber. Measurements were carried out by filling the test cell with a known volume of saline or blood and then increasing or decreasing the volume as indicated above. Resistance measurements were obtained by rebalancing the bridge using the recorder as a null point indicator. The results of 5 sets of experiments are summarized in Table I. It can be seen that wide variations from a perfect cylinder produce only small errors in the calculated volume change.

Table I. Relation Between Small Volume Changes and Impedance Changes

R (ohms)	△R (ohms)	V(c.c.)	△Vc.c.	△V (cal.)
196	11	3.36	0.2	0.19
185	10.5	3.5	0.2	0.20
174	9	3.7	0.2	0.19
166	8	3.96	0.2	0.19

Data from mechanical model showing good agreement between volume change calculated from resistance measurements and actual volume change.

The measurement of volume changes in a system containing flowing blood introduces a possible source of error in the variation of conductivity with change in mean velocity.[1] The relative magnitude of this error has been evaluated in the mechanical model. Pulsatile inflow was obtained with a Wesolowski type perfusion pump which produces a volume pulse of known amount. When the stopcock (*E*) on the water jacket of the test cylinder is closed, the volume of the rubber chamber is fixed and any impedance change will result entirely from change in specific conductivity due to change in velocity. The tracing obtained in such an experiment is shown in Fig. 3. The arrow indicates the point of conversion to a rigid system. It will be seen that a small but definite deflection occurs, indicating a small magnitude change due to velocity alteration.

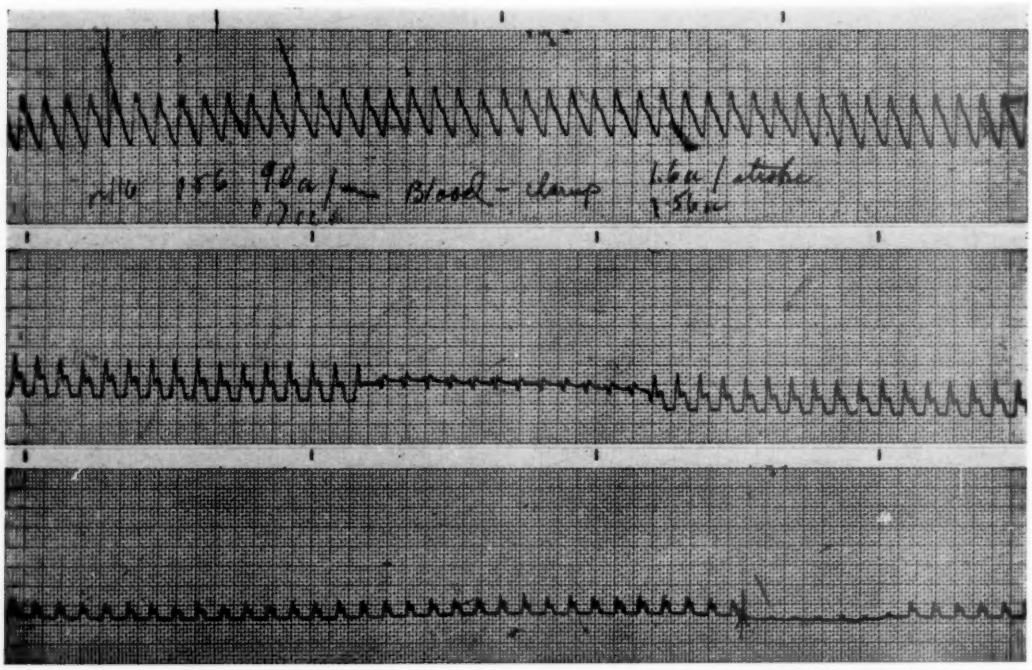

Fig. 3.—Impedance curves from mechanical model. Lower curve taken with outflow unconstricted. The arrow indicates conversion to a rigid system and no further volume change. Middle curve obtained with slight outflow constriction. Upper curve with outflow constricted sufficiently to prevent complete emptying of test cell between pulses.

The mechanical model can be modified to simulate a vascular bed by applying an artificial peripheral resistance in the form of a constricting clamp on the outflow from the test chamber. Fig. 3 illustrates a tracing from the model with the clamp in place. The volume change is measured directly by the change in water level in the calibrated burette. Comparison of this value with the figure obtained by calculation from the impedance data reveals a close agreement (0.7 c.c. versus 0.68 c.c.). It is concluded that the instrument will follow pulsatile volume changes in a homogeneous system with considerable precision.

Although good agreement between relative resistance change and relative volume change was obtained on a mechanical model, it does not follow that equally good results would necessarily occur for measurements on an intact limb. Current flow in the model was purely resistive in character whereas pronounced capacitive impedance exists in biologic tissues. With the instrument described, it is possible to balance out the capacitive reactance so that only change in resistance is recorded. These resistance changes were compared to absolute volume change by simultaneous measurement with a sensitive air plethysmograph in a constant temperature room. The agreement between calculated and actual volume change was about 10 per cent. It is interesting that van den Berg[7] attempted to make a similar

comparison with the finger plethysmograph and found that the impedance data were related to the actual volume change by a factor of 0.67. When the value for capacitive reactance as measured during our study was used to calculate the total impedance vector, we obtained a corresponding factor of 0.69. It seems likely, therefore, that the lack of agreement between impedance and mechanical plethysmography reported by previous authors was a result of the error introduced by the effect of the uncompensated capacitive reactance.

When volume change was calculated from impedance changes following venous occlusion and compared with the actual volume change observed in the air plethysmograph, the agreement was less satisfactory. The impedance plethysmograph provided volume data consistently lower than the actual volume change. Our studies have indicated that venous occlusion does not alter the over-all capacitive reactance and therefore the discrepancy is probably due to a change in specific resistance which results from a shift of plasma to tissues and consequent alteration of the resistivity of the blood.[7]

ESTIMATION OF BLOOD FLOW

An accurate, independent measurement of blood flow through an extremity is essential for determining the validity of the impedance plethysmograph. Total venous diversion was selected as the most reliable method available.

The method consists of disarticulation of a dog's leg at the knee, preserving only the popliteal artery, vein, and nerve. All muscles are divided at their origin or insertion and the cut ends controlled with suture ligatures. A "T" cannula is placed in the popliteal vein close to the volume of tissue under study. By means of pinch clamps, venous blood can return to the animal or be diverted into a graduate cylinder for measurement of flow.

The present studies have shown that for small volume changes (Δv), the following relation is valid: $\frac{\Delta v}{V} = \frac{\Delta r}{R}$ where Δr is the measured resistance change due to Δv and where V and R are the total volume and resistance of the leg, respectively. The application of this formula requires a measurement of the total resistance of the leg (R) which is read directly from the bridge. Δr is obtained from the record and the total leg volume (V) is obtained at the end of the experiment by immersing the leg in water and measuring the volume of water displaced.

With accurate information as to the volume change with each pulse beat, attention was directed toward applying these data to the calculation of blood flow. The method of approach was to attempt to isolate inflow from outflow by an analysis of the pulse contour. It is generally accepted that venous outflow is quite constant throughout the entire pulse cycle so that, if this quantity could be inferred during any portion of the cycle, total venous outflow would be known. The initial method of analysis was empirical and consisted first of the direct measurement of venous outflow in the disarticulated leg. Using these data, a venous outflow curve was constructed and superimposed on a simultaneously observed volume pulse (Fig. 4, *A*). In each case, the outflow curve fits the straight, descending, late diastolic limb of the volume curve. The implication of this observation was that the total volume change of a large segment of the leg during diastole was due principally to venous outflow or, to put it in a slightly different way, arterial inflow was close to zero during this phase of the

pulse cycle. This does not imply that arterial flow through the small vessels within the segment of leg under study fell to zero but only that no appreciable quantity of blood entered that portion of the leg from the aorta during late diastole. There is considerable corollary evidence in the literature to support such a concept. The pressure studies of Hale[3] suggest that in the distal arterial tree diastolic flow is probably close to zero.

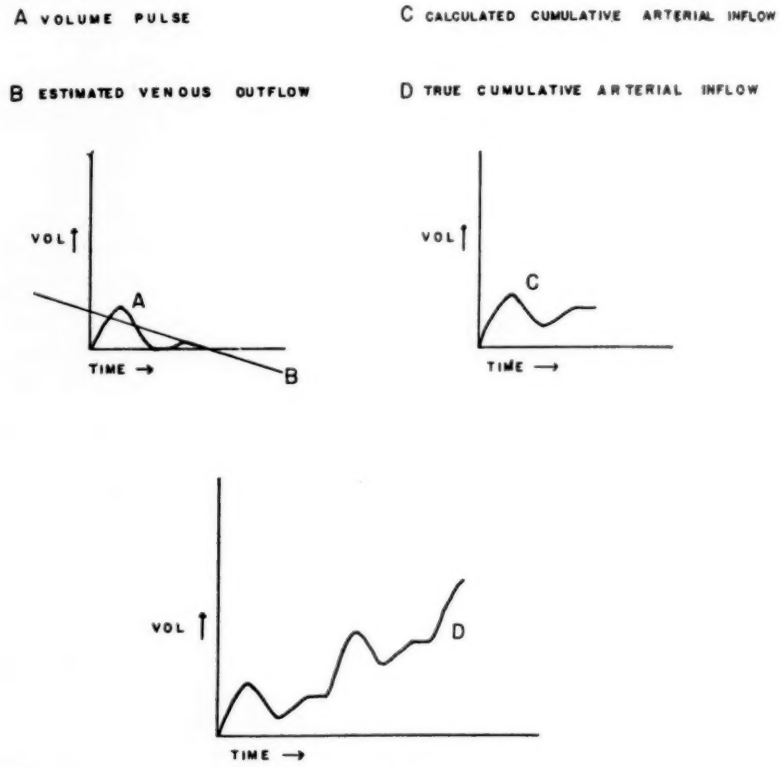

Fig. 4.—*A*, Method of estimating venous outflow. *B*, Calculated curve derived by assuming zero venous outflow. *C*, Experimental curve following venous occlusion. *D*, True cumulative arterial inflow.

The concept that the limb volume change in late diastole is a measure of venous outflow has been verified in the disarticulated hindlimb of the dog. Under these circumstances occlusion of the isolated popliteal vein provides instant and quantitative cessation of venous outflow. If the above hypothesis is correct, there should be no change in volume of the limb during late diastole and therefore the slope of the volume curve should be zero. Fig. 4 illustrates this type of study and confirms the suggestion that there is negligible arterial inflow during this phase of the cycle. It would appear from this experiment that the slope of the diastolic portion of the curve in a nonoccluded limb results entirely from venous outflow and is therefore a measure of total limb blood flow. Simultaneous comparison of directly measured limb blood flow with the estimated blood flow based on impedance data shows an agreement of about 10 per cent. The data and method of calculation from a sample experiment are shown in Table II.

TABLE II. DETERMINTION OF BLOOD FLOW

EXPERIMENT	SLOPE* (MM./SEC.)	△r (OHM/SEC.)	△V (C.C./SEC.)	FLOW (C.C./MIN.)	OBSERVED FLOW (C.C./MIN.)	COMMENT
1	130	0.52	0.342	20.5	21.4	Control
2	115	0.46	0.303	18.1	16.9	Control
3	62	0.25	0.163	9.8	8.9	(1 minute after 0 1 mg. Adrenalin)
4	43.5	0.17	0.114	6.8	6.4	(5 minutes after 0.1 mg. Adrenalin)

Sample data sheet from simultaneous impedance plethysmograph and direct blood flow determinations.

Leg volume 250 c.c. Total resistance (R) 380 ohms. Calibration 0.1 ohm = 25 mm. Flow = $\triangle v/mm. = \frac{V \triangle r}{R} \times 60$.

*Average of 10 consecutive pulses.

CLINICAL APPLICATION

The technique of impedance plethysmography has been applied to the study of the effects of the sympathetic nervous system on the development of collateral circulation. In a group of 12 dogs, collateral circulation to the legs was induced

TABLE III. THE EFFECT OF SYMPATHECTOMY ON THE DEVELOPMENT OF COLLATERAL CIRCULATION

DOG NUMBER	LIMB TESTED	LIGATION OF MIDDLE SACRAL		1 WEEK	2 WEEKS		5 WEEKS	7 WEEKS
		BEFORE	AFTER					
1	Right	*	*	*	Died			
	Left	6.0	*	*				
2	Right	8.4	*	4.2	Died			
	Left	8.4	*	7.2				
3	Right	Died						
	Left							
4	Right		*	*	9.6	9.3	11.4	16.6
	Left		*	9.0	16.2	14.2	19.5	18.0
5	Right					8.7	15.6	15.0
	Left				10.2	18.0	19.8	10.0
6	Right						14.3	19.0
	Left						20.4	17.0
7	Right	8.2	6.0		9.5		10.0	18.0
	Left	21.4	3.0		14.3		18.0	34.0
8	Right	12.0	8.4		5.5		7.8	19.0
	Left	12.0	14.0		20.0		9.6	36.0
9	Right	13.8	*		7.5		12.0	20.0
	Left	10.8	*		12.0		19.2	24.0
10	Right	7.6	*		16.5		17.5	24.0
	Left	11.7	*		16.3		22.5	35.0
11	Right	16.0	*		12.0		8.0	
	Left	20.4	*		13.7		20.0	

Bilateral iliac ligation and right lumbar sympathectomy carried out in all animals. Middle sacral artery ligated 2 weeks later.

Blood flow shown in cubic centimeters per minute per 100 Gm. of leg.

*Insufficient for calculation.

by ligation of both common iliac arteries. At the same time a right lumbar sympathectomy was performed. Immediately following this procedure, there was a marked fall in blood flow to both legs with no detectable difference between the control and sympathectomized limbs. Improvement in blood flow due to the development of collateral circulation was relatively rapid, and, in about 2 weeks, flow had returned almost to control levels in both legs. The adequacy of the collateral circulation was then tested by ligating the middle sacral artery, the principal source of collateral circulation to the lower extremities. Following this procedure, there was an immediate fall in the blood flow to both legs, but the fall was most marked in the sympathectomized limb. Serial blood flow measurements carried out during the ensuing 7 weeks indicated a consistently higher limb blood flow in the leg with normal sympathetic innervation (Table III). These studies suggest that lumbar sympathectomy may interfere with the development of collateral circulation following acute arterial occlusion in the dog.

DISCUSSION

The application of resistance measurements for the determination of blood flow has been considered by several investigators.[5-7] Although the form of the impedance curves has been repeatedly demonstrated to be identical with those obtained from mechanical plethysmography, a quantitative comparison could not be made because of insufficient data concerning the electrical properties of biologic tissues. The present studies have been carried out using standard electrical circuits to determine the precise nature and values of these properties. Limb volumes calculated from these data were then found to agree well with direct measurements of both volume and limb blood flow.

A further limitation of both impedance and mechanical plethysmography has been inadequate sensitivity for obtaining pulse tracing of sufficient magnitude to permit pulse analysis, especially from measurements on the lower leg. Measurements have usually been carried out on the toes because there is a high ratio of pulse volume to total volume and therefore a less sensitive instrument provides adequate curves. This type of study provided a good index of skin blood flow but did not include muscle blood flow. Flow through the latter vessels may respond quite differently from the skin in response to medical and surgical therapy. The present technique provides pulse waves from the calf which are of sufficient amplitude to permit quantitative analysis of muscle blood flow.

The instrument described has the practical advantage of having been constructed from standard, readily available components. It is likely, however, that it could be greatly simplified and miniaturized for routine clinical application. The results reported on the effect of sympathectomy were carried out on the dog, and it is not our intent to suggest that similar results would necessarily occur in the intact human extremity. Further studies in the human should provide valuable information concerning the role of sympathectomy in the treatment of peripheral vascular disease.

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Discussion

DR. THOMAS C. MOORE (Muncie, Ind.).—Certainly, the electronics of the impedance plethysmograph were much too complicated for my comprehension. However, we were fortunate at the Indiana University Medical Center to have with us an electronics consultant who aided us in the studies which we carried out on this subject. Dr. Shumacker and his associates published their work with the impedance plethysmograph on clinical studies in 1952 in the *Archives of Surgery* and found this device quite helpful in correlating problems in peripheral vascular disorders and comparing those with normal controls.

In the past 2 years, we have been interested in transferring the usefulness of this instrument to the dog laboratory. In evaluating certain problems, we have found the impedance plethysmograph quite helpful. Its sensitivity has aided us a great deal and in certain respects it may be more valuable than the ordinary mechanical plethysmograph, especially in studying the peripheral circulation of dogs.

I would like to ask Dr. Powers what type of electrodes he used. In patients, we used 2 electrodes, 2 on opposite sides of the proximal phalanx and 2 on opposite sides of the distal phalanx. This proved somewhat cumbersome in dogs so we used 2 concentric copper electrodes. These gave very sensitive recordings.

I would like to show an illustration of the results we were able to obtain in the dog using the impedance plethysmograph. In the first slide the arrow illustrates the point at which we carried out acute occlusion of the femoral artery proximal to the area which we were studying. Note the very dramatic disappearance of the wide oscillations.

(Slide.) Our principal interest in this study was evaluating the peripheral vascular response to intra-arterial injection of radiopaque media. One of our main problems here is the massive spasm which one encounters. The upper tracing shown here represents the contralateral limb recording through a pressure transducer. A polyethylene tube was introduced into the contralateral artery as a control simply to illustrate that the changes that we encountered in the injected limb were due to local rather than to systemic changes. These were fairly constant throughout.

Here is the impedance plethysmographic tracing. (Slide.) In the lower part is the control. This is acute mechanical occlusion of the artery. Here it is with an injection of Uretan used to increase the vasodilatation. We interpret it that one encounters it quite initially. This is when the first amount of Uretan strikes the peripheral arterial tree. For a few moments, there is a vasodilating effect. However, quite promptly a massive vasoconstriction occurs showing almost a complete lack of any possible blood flow in that area.

(Slide.) We carried out a number of studies using this modality and found that massive injection again here shows massive spasm. However, we found that repeated injections with large doses produce progressively less arterial spasms and, finally, about the fourth and fifth injection of large amounts of radiopaque metauricon in this case, a vasodilatation effect occurred.

Small doses produced the vasodilating effect and repeated small injections of an otherwise vasoconstricting substance protected against a subsequent massive injection and the vasoconstriction that one would expect in this circumstance.

In addition, we supplemented the results of Dr. Grimson's group study which was reported recently in SURGERY with the discovery that the simultaneous injection both of crystalline and procaine did not protect against this vasoconstricting effect but that the prior injection of procaine had a very significant and dramatic protecting effect against this very serious arterial spasm encountered with almost every type of arterial technique that one chooses to employ.

DR. FIORINDO ANTHONY SIMEONE (Cleveland, Ohio).—We considered the possibility of this type of plethysmography some years ago and gave it up because we felt we were not sufficiently expert electronic engineers. We chose the type of plethysmograph which converts mechanical changes to changes in electrical energy because they were easier to manage and amplify, at least with our experience.

In the impedance type of plethysmograph, one is skimming off the top of the relatively high resistance which you must start with, and electronic engineers tell me that the amplification of that type of signal is a little more difficult than the other.

In regard to the late effects of sympathectomy in the development of collateral circulation, were the postoperative determinations in the dogs done under anesthesia? If they were then the results are exactly what you would expect, because the vascular resistance in the sympathectomized limb some days after operation—that is, after sympathectomy—will be very much higher than the vascular resistance of the sympathetically intact limb under anesthesia.

DR. POWERS (Closing).—First, I would like to make one correction of a misapprehension. The co-author is not Dr. Schaffer. He is a Mr. Schaffer and he is an electronics wizard of the greatest magnitude. Otherwise, we would never have tackled this problem, I assure you.

As far as the question of electrodes is concerned, we have tried to make the whole thing as simple as possible for clinical use and, because we wished to carry out measurements on the whole lower leg rather than just a finger or a toe, we used the standard ECG rubber strips that are used for holding on electrodes and threaded a piece of stainless steel wire through them. We put on some electrode paste which made them very easy to attach to the skin, just as with electrocardiogram.

I would like to emphasize that it is a simple thing to do. All you must do to the patient is wrap 2 wires around his legs and the actual operation of the instrument is fairly simple.

In response to Dr. Simeone's question, the measurements were not carried out under anesthesia. They were carried out over a period of 8 weeks following the ligation of the middle sacral artery. By 8 weeks, the blood flows were quite comparable in the 2 legs, so that the difference which we observed as the result of sympathectomy lasted for perhaps 2 or 3 weeks. By then, the blood flow was back completely to normal in both legs and there was no further difference.

A VALVE MECHANISM TO PREVENT GASTROESOPHAGEAL REFLUX AND ESOPHAGITIS

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THE cardioesophageal area is normally competent in preventing reflux of gastric secretions into the esophagus when the stomach is maintained in its normal position below the diaphragm with the terminal esophagus bordered by a snugly fitting diaphragmatic hiatus. The incompetence associated with a sliding hiatal hernia is automatically corrected when the now fairly standardized methods for replacing the stomach below the diaphragm and correcting the hiatal defect are applied. Unfortunately, it may not be possible to restore this normal anatomic arrangement when the esophagus is short, fibrosed, or strictured, or when plastic and excisional surgery of the distal esophagus and cardia are performed.

When the cardia remains above the diaphragm, a situation resembling a hiatal hernia with incompetence results. It has only recently been appreciated that reflux esophagitis and its disabling complications are common sequelae. The problem that presents, therefore, is how to establish continuity between the shortened esophagus and stomach in a way that will prevent gastroesophageal reflux and esophagitis. Some of the experimental and clinical operative procedures designed to prevent reflux esophagitis may be listed as follows: interposition of a segment of jejunum or colon between esophagus and stomach,[4, 17, 22, 27, 31] extensive esophagectomy and high esophagogastrostomy,[29, 30, 32] various forms of gastric resections, gastric drainage procedures and vagotomy used alone or in combination,[7, 13-16, 19, 20, 33] advancing the shortened esophagus from the hiatus to the dome of the diaphragm,[12, 23] reconstitution of the cardioesophageal angle,[6, 8, 9, 26, 28] and construction of cardioesophageal "valves."[11, 21, 24, 34-36]

With this problem in mind, the present study was undertaken in an effort to find a simple procedure for maintaining a competent esophagogastric union independent of its position in relation to the diaphragm. A lead was taken by recalling that certain other tubular structures such as ureter and common bile duct have an oblique course through the muscular wall before entering their hollow distensible viscera. Although we found it possible to create a tunnel for the esophagus between the muscular and mucosal layers in specimens of human and dog stomachs, the disproportions in size between the esophagus and stomach wall plus the bulkier contents transported through the esophagus

This investigation was supported in part by the Research and Development Division, Office of the Surgeon General, Department of the Army, under Contract No. DA-49-007-MD-750.

Read at the Annual Meeting of the Society of University Surgeons, Boston, Mass., Feb. 13-15, 1958.

made this approach appear to be less attractive. Instead, the principle used in the Witzel enterostomy was modified by burying the terminal esophagus in a loose tunnel formed from the redundant fundus. The problem was also approached by partially encircling the terminal esophagus with a sling of knotted nylon net* which was sutured back onto both surfaces of the fundus. Our experiences with these methods and their modifications in dogs will be presented in this paper, along with pertinent observations on human and canine specimens of stomach and esophagus.

MATERIALS AND METHODS

Part I.—Thirty normal fresh post-mortem human stomachs with attached esophagi were studied initially. A radiopaque surgical sponge strip marker was sutured along the right esophageal border and lesser curvature of the stomach; another similar strip was sutured to the left esophageal border, over the fundus, and onto the greater curvature. A Foley catheter was then passed through the pylorus and the stomach filled with water. A side arm allowed intragastric pressure measurements. Reflux was noted by flow from the open esophagus. In some specimens a catheter was fixed in the distal esophagus for recording simultaneous pressures.

The specimens were first checked for gastroesophageal reflux in different positions and with variations in the measured angle between the esophagus and fundus. The esophagogastric angle was then reconstructed and even exaggerated by apposing the fundus to the left esophageal border using a single row of interrupted sutures for varying distances of 4 to 10 cm. The tests for reflux were again repeated with and without the opposite right border of the esophagus stabilized or fixed against an immovable object. For fluoroscopic study a paraffin board was made, and the right border of the distal esophagus stabilized against a protruding row of straight pins.

After re-creating the esophagogastric angle as described, two additional procedures were used. In one the distal esophagus was buried in tunnels of varying lengths that were formed by overfolding the fundus around the esophagus using a single row of interrupted sutures. In the second procedure, a strip of nylon net 2 to 4 cm. wide was placed around the right border and sides of the terminal esophagus and anchored by sutures to the anterior and posterior surfaces of the fundus. This sling never completely encircled the stomach. All specimens were checked for reflux and examined by fluoroscopy. The esophagogastric junction and valve area were inspected from the stomach through a lighted hollow metal tube with a glass eye piece and from above through an esophagoscope.

Part II.—Fifty adult mongrel dogs were studied. The abdomen and left chest were opened through a thoracoabdominal incision. After completely dividing the diaphragm and crus down to the esophagus, a Foley catheter was inserted into the stomach through an incision at the pylorus. Another Foley catheter had been placed through the mouth and left in the upper thoracic esophagus. Both catheters were connected to saline manometers. The proximal stomach and distal esophagus were freed so that the cardioesophageal junction and fundus could be moved into the left chest. Radiopaque strips were sutured to the right and left borders of the esophagus and stomach as described for the specimens. Reflux was determined in all animals by filling the stomach from the pyloric end with sterile saline, exerting manual pressure on the middle of the stomach, observing the shape of the distal esophagus, and carefully noting the intragastric and intraesophageal pressures simultaneously. The divided diaphragm was then closed loosely around the stomach below the esophagogastric junction, the chest incision was approximated, and the pressures were observed again as manual pressure was applied to the stomach.

The animals were divided into 5 groups (Fig. 1). For controls, the cardioesophageal junction was placed above the diaphragm simulating a hiatal hernia and checked for competence. This was done on most dogs before constructing the angle or valve; two were left

*Nylon B, kindly supplied by Ethicon, Inc., Somerville, N. J.

in this position above the diaphragm for further study. A group of 6 dogs had the cardioesophageal angle reconstructed above the diaphragm by approximating the fundus to the left esophageal border with a row of interrupted sutures for a distance of 4 to 8 cm. from the cardioesophageal junction. With the angle reconstructed, the next group of 20 dogs had the distal esophagus buried in a tunnel of fundus 2 to 4 cm. long and loose enough that one finger could be easily inserted under the tunnel along the esophagus. Another group of 20 dogs had a sling of nylon net placed around the distal esophagus onto the stomach after the esophagogastric angle had been reconstructed. The band of net in contact with the distal esophagus was about 3 cm. wide. Although the part of the net on the esophagus was kept the same size, later in the study the distal limbs of the sling were fanned out to a butterfly shape rather than the original uniform ribbon shape so as to obtain more purchase on the fundus. The net was held in position on the right esophageal border by 2 sutures, but the sling was left loose enough so that one finger could be inserted along either side of the esophagus and fundus when the stomach was not fully distended. Approximately half of the animals had the distal esophagus and proximal stomach resected, followed by a standard 2-layer esophagogastric anastomosis before the angle was reconstructed and a fundus or sling tunnel made. Animals with an esophagogastric anastomosis had a concomitant pyloromyotomy.

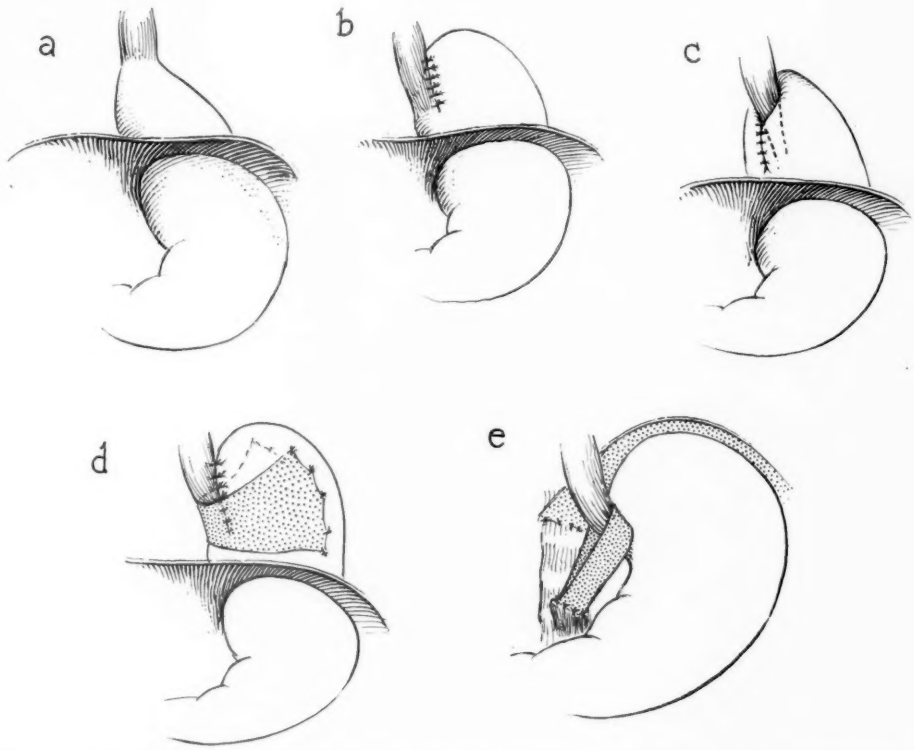


Fig. 1.—Diagrammatic representation of the various procedures. *a*, Cardioesophageal junction above diaphragm simulating a hiatal hernia. *b*, Reconstructed cardioesophageal angle alone. *c*, Fundus tunnel. *d*, Plastic sling tunnel. *e*, Nylon diaphragm replacement with plastic crus.

Two dogs, actually part of another study,[2] had the entire left hemidiaphragm and crus excised and replaced by a sheet of knotted nylon net. The stomach was positioned below this net, and the distal esophagus was angulated posteriorly and to the right by a separate 2.5 cm. wide strip of nylon net that was looped around the esophagus in the same position as the original left crus. The ends of this nylon crus were sutured to the remnants of the original muscular crus on the vertebrae. This nylon crural loop remained below the nylon diaphragm.

In all animals the esophagogastric junction, angle, and tunnels were left in the chest with the diaphragm loosely sutured to the stomach below. The diet was gradually increased

from fluids to full kennel ration. Those with esophagogastric anastomoses received nothing orally for the first 3 postoperative days. Careful records were kept of weight and eating habits.

Esophagoscopy was routinely performed under Pentothal anesthesia between 4 and 6 weeks and before autopsy. After the first esophagoscopy, a nasogastric tube was inserted through the esophagogastric junction and 200 c.c. of thin barium injected into the empty stomach. Reflux was checked under fluoroscopy on a tilt table using the Trendelenburg position and manual pressure on the abdomen. Barium was injected through an inflated Foley catheter then placed in the upper thoracic esophagus, and its course was observed through the esophagus into the stomach. The amount of pressure needed to accomplish this passage was also noted. Selective spot x-rays were taken.

Fourteen dogs (2 angles alone, 5 fundus tunnels, 5 sling tunnels, and 2 with nylon diaphragms and crura) were given intravenous apomorphine while awake to induce vomiting. All were in a fasting state and were allowed to drink either 200 c.c. of milk or thin barium before apomorphine administration, depending upon whether or not they were to be observed fluoroscopically.

At autopsy gastric and esophageal pressures were measured as described at operation with the stomach and esophagus in position and again when they were removed from the body. Specimens were carefully examined for valve function, stenosis, and esophagitis. A few animals that died of unrelated causes did not have either esophagoscopy, barium studies, or apomorphine; however, they were checked for competence at post mortem.

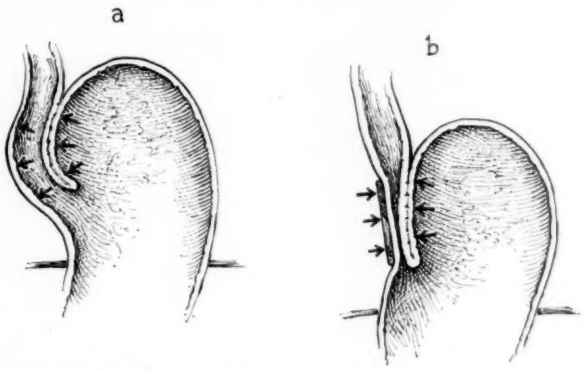


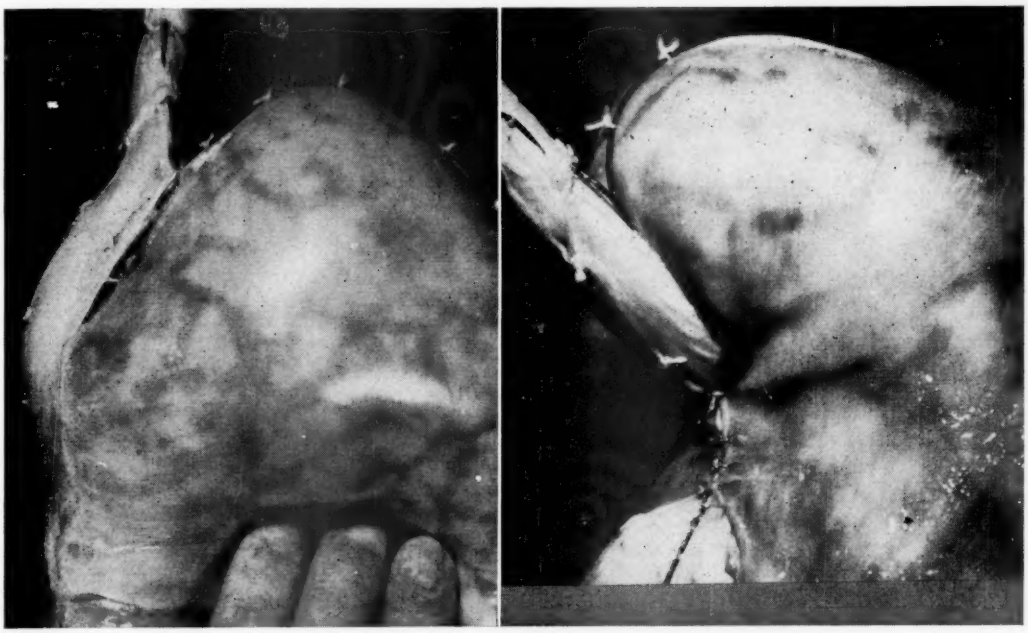
Fig. 2.—Diagrammatic representation of reconstructed angle alone. The bulging right border of the distal esophagus (*a*) prevents competence unless it is properly buttressed (*b*).

RESULTS

No gross anatomic sphincter was apparent in the distal segment of the 30 human esophagi studied, although in one the circular muscle was locally hypertrophied for no obvious reason. Histologic examination of the distal esophagus was of limited help in searching for evidence of an anatomic sphincter, since slight variations in the angle at which the esophageal wall was transected could present the misleading appearance of thickened muscle. There was no evidence of an anatomic mucosal flap or valve.

It has been stated that there is increased resistance to the passage of fluid from stomach to esophagus in the isolated specimen in contrast to that seen when fluid passes from esophagus to stomach and that, if the esophagogastric angle is maintained, flow from stomach to esophagus does not occur.[3, 5, 10, 25] In our specimens fluid flowed just as easily from stomach to esophagus as in the reverse direction, even when the esophagogastric angle was reconstructed. By the use of radiopaque strips we could demonstrate in specimens and in many of the living dogs that with the angle reconstructed the fundus caused

the left border of the esophagus to bulge to the right into the esophageal lumen (Figs. 3 and 4). Unless the opposite right wall of the distal esophagus was stabilized against a nonmovable object such as a block or a row of pins, it too bulged to the right resulting in a curved but open distal esophagus. In



A. B.

C.

Fig. 3.—Specimen of human stomach and esophagus with black radiopaque strips sewn in place. Cardioesophageal angle reconstructed in all specimens by suturing fundus to esophagus. *A*, Angle alone—incompetent with distal esophagus bulging to the right. *B*, Close-up oblique view of competent fundus tunnel, overdistention of stomach exaggerated by extreme manual pressure. *C*, Competent plastic sling tunnel.

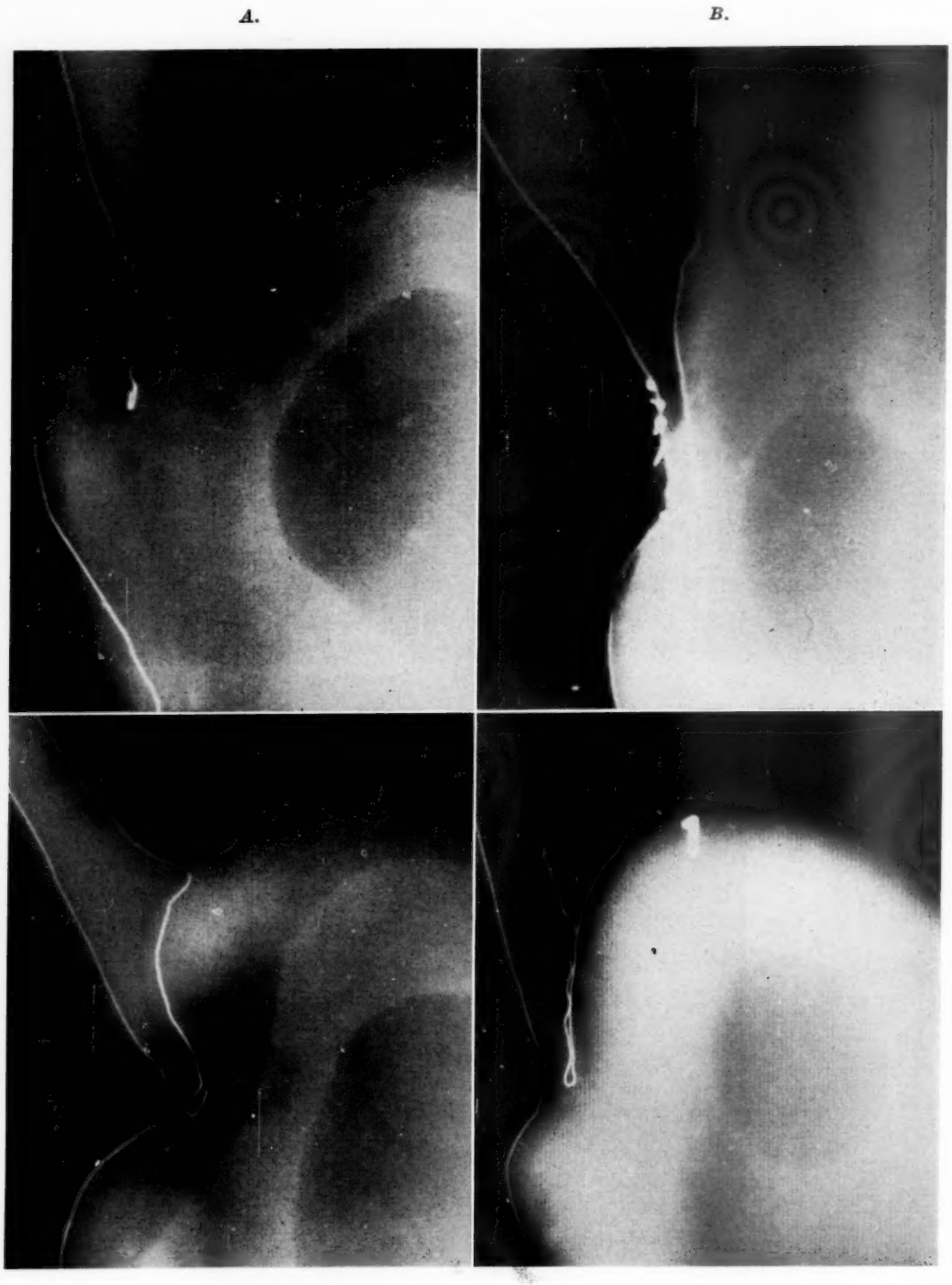


Fig. 4.—X-rays of distended human stomach with esophagus specimens, all with cardio-esophageal angle reconstructed. Radiopaque strips appear white and outline left and right borders of distal esophagus and stomach (see text). *A*, Angle alone; note incompetent bulging distal esophagus. *B*, Angle alone; competent because right border of esophagus buttressed against row of straight pins protruding from paraffin board. *C*, Competent fundus tunnel. *D*, Competent plastic sling tunnel; in this specimen double radiopaque strip used between fundus and esophagus (stomach distended with water, air bubbles account for density variations).

only one of the 6 dogs in which only the esophagogastric angle was reconstructed was there competence on esophagoscopy and fluoroscopy. No reflux occurred in this dog because the distal esophagus had become positioned between the fundus and vertebrae. When the specimen was removed from the body and the right esophageal border no longer maintained against a fixed surface, gastroesophageal reflux occurred.

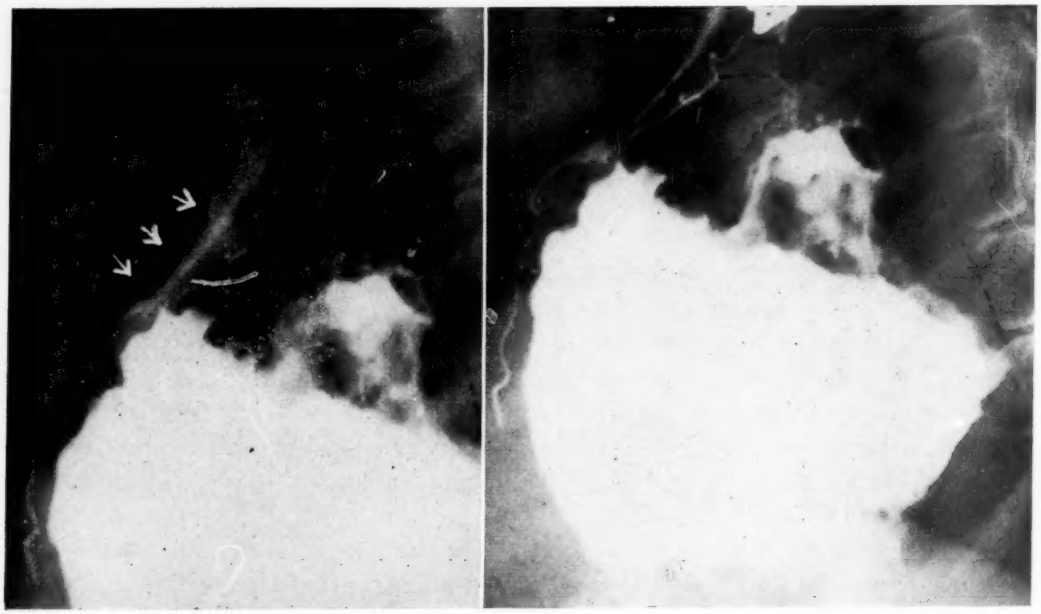
Simulating a hiatal hernia by placing the cardioesophageal junction above the diaphragm and permitting the freed esophagus to shorten by virtue of its longitudinal muscle always resulted in incompetence. It was of interest that in a number of animals there was an initial resistance to reflux from stomach to esophagus until the rising intragastric pressure reached a certain level, occasionally up to 10 cm. H_2O. When this critical point was passed, the competence suddenly broke as indicated by a rise in intraesophageal pressure equal to that in the stomach; there was then free reflux with no further resistance. All animals with fundus tunnels and sling tunnels were competent. Many lost weight during the first several weeks but all regained their preoperative weights and had no difficulty eating. In the beginning, several dogs with fundus tunnels constructed too tightly had several weeks of regurgitating freshly gulped solids.

The esophagoscope dropped readily into the stomachs of dogs with hiatal hernias and reconstructed esophagogastric angles. In those with the fundus tunnels, sling tunnels, and nylon crura, compression and slight-to-moderate resistance was encountered in the distal esophagus. With a little pressure the esophagoscope popped through this area into the stomach, occasionally followed by an escape of gas or fluid if the stomach was full. All fundus and sling tunnels were competent with barium studies whereas the hiatal hernias and reconstructed angles had reflux except for the one dog described. Fluoroscopic observation of the radiopaque strips in fundus and sling tunnels showed the 2 walls of the distal esophagus to be approximated when preventing reflux and separated when letting liquid pass (Fig. 5). It required surprisingly little pressure to inject the thin barium mixture through the esophagi into the stomachs of anesthetized dogs with fundus and sling tunnels.

Intravenous apomorphine given to unanesthetized animals always caused regurgitation of milk and barium from the stomachs of those with hiatal hernias and reconstructed angles. Those with fundus and sling tunnels had violent retching and frothy salivation but never actual regurgitation. One with a somewhat loosely placed but competent sling tunnel had some barium regurgitate into the esophagus fluoroscopically with violent retching. It was of special interest that the 2 with diaphragm replacement and nylon crura had regurgitation although they were competent at esophagoscopy, fluoroscopy, and post-mortem examinations. Unfortunately, the only competent animal with a reconstructed angle alone was accidently sacrificed before being tested with apomorphine.

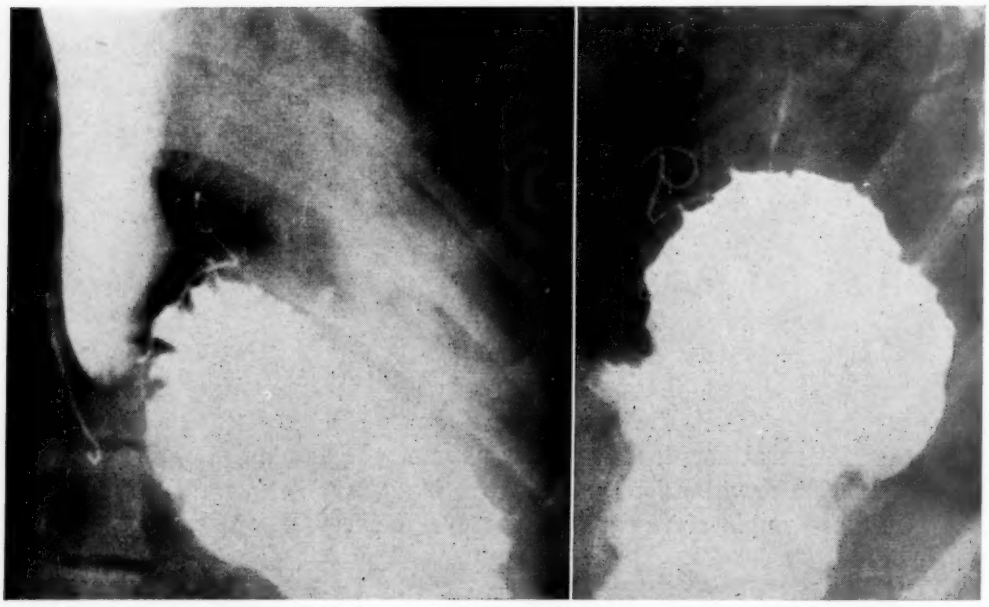
Examination of the inside of human and canine stomachs through the lighted tube revealed a slitlike cardioesophageal orifice with the fundus and sling tunnels. The esophageal orifice was difficult to find among the gastric rugae without the help of a slender probe projecting into the stomach from the

esophagus. With construction of the cardioesophageal angle alone, it was possible to see into the lumen of the partially open distal esophagus. With the stomach distended and the upper esophagus clamped to prevent gastric



A. *B.*

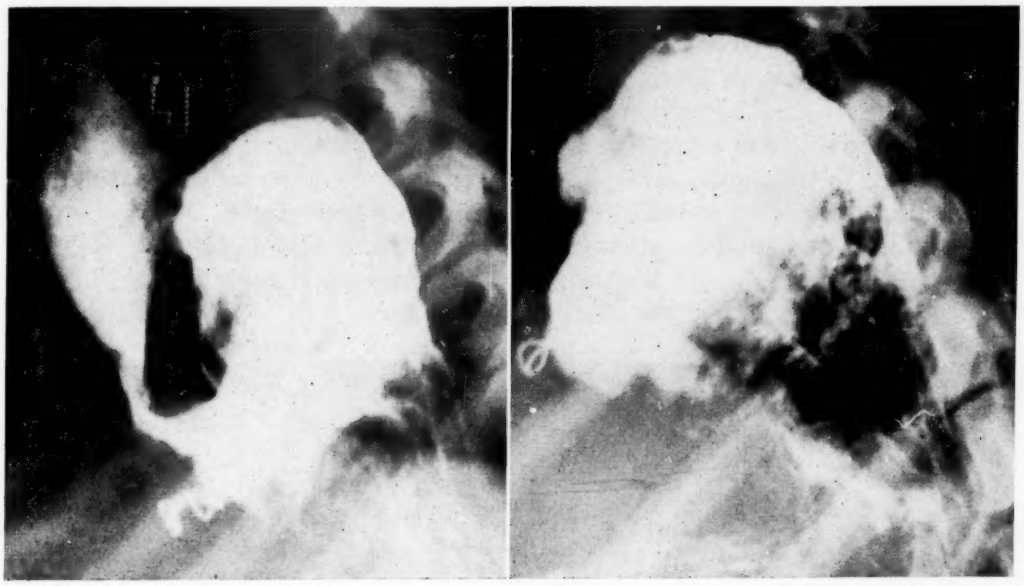
Fig. 5.—X-rays of dog 2 months after esophagogastrostomy and sling tunnel. Oblique spot films. Broken line outlines fundus not completely filled with barium; arrows point to radiopaque strips outlining walls of distal esophagus. *A*, Distal esophagus open as barium flows into stomach. *B*, Esophagus walls approximated preventing reflux from stomach with manual abdominal pressure.



A. *B.*

Fig. 6.—X-rays of dog with sling tunnel 3 months after operation. Oblique spot films. *A*, Esophagus widely open as barium runs into stomach. *B*, No reflux with extreme Trendelenburg position (see text).

deflation through the esophagus, the inward bulging left wall of the esophagus due to the fundus and outward curve of the right esophageal wall could be partially visualized from the stomach.



A. *B.*

Fig. 7.—X-rays of dog with fundus tunnel 2 months after operation. *A*, Barium injected into stomach from esophagus. Paraesophageal part of fundus incompletely filled with barium at this instant effectively showing the distal esophagus. *B*, No reflux with abdominal pressure and Trendelenburg position.

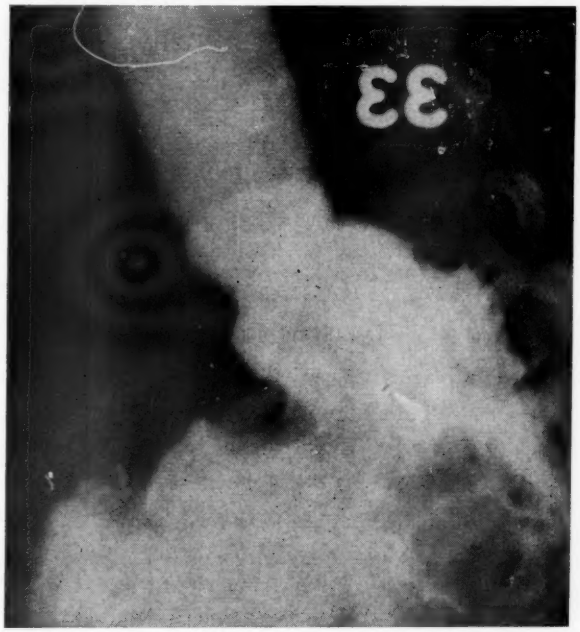
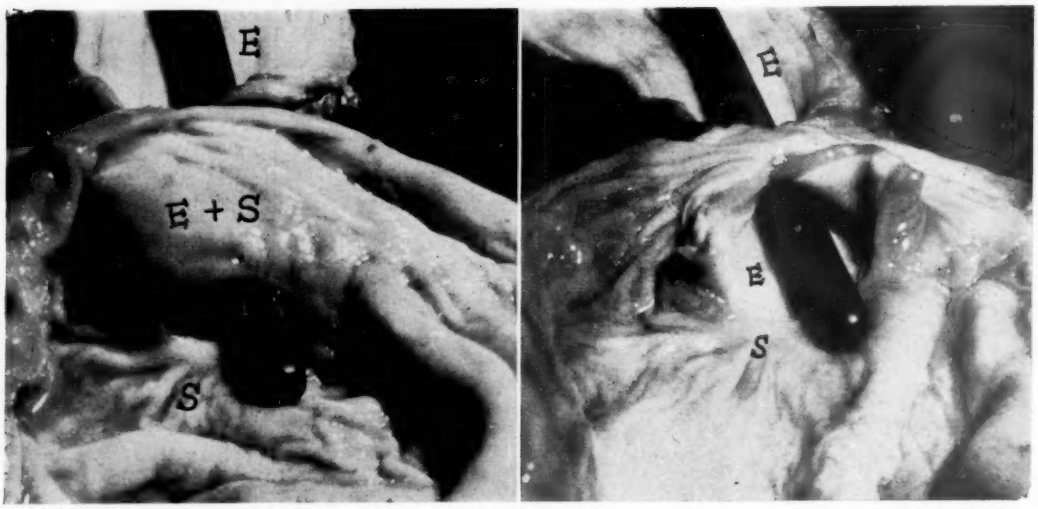


Fig. 8.—X-ray of dog with reconstructed cardioesophageal angle alone, 2 months after operation (see text). Moderate abdominal pressure causes free reflux from stomach into esophagus. Fundus above diaphragm only partially filled with barium.

Two dogs could not be checked for competency at autopsy. One with an esophagogastric anastomosis and fundus tunnel died from necrosis of the distal esophagus due to a technical error. The other with an esophagogastric anastomosis and sling tunnel done early in the study died one month postoperatively from a 1 cm. perforated peptic ulcer in the right posterolateral esophagus at the line of anastomosis. The probable explanation is that the sling was placed too high on the esophagus, leaving esophageal mucosa below the valve exposed to gastric juice. Careful attention to more accurate placement of the plastic sling extending to the lesser curvature appears to have prevented recurrence of this catastrophe. Esophagitis, otherwise, was not seen in animals followed as long as 2 years. Two other animals, one with a fundus tunnel and the other with a sling tunnel, competent by all tests, died from small bowel obstruction due to adhesions and volvulus. In both, the stomach was greatly distended but the valve remained competent.



A. *B.*

Fig. 9.—Stomach (*S*) and esophagus (*E*) from dog with plastic sling tunnel autopsied 6 months after operation. Close-up view of tunnel looking inside the stomach after opening the greater curvature and also the left border of the esophagus above. Black rod projecting from esophagus into stomach lies in the tunnel. Plastic sling on outside not visible. *A*, Orifice of tunneled esophagus into stomach partially opened by the protruding rod. Esophageal mucosa was not visible from stomach when tunnel walls approximated. *B*, Inside wall of stomach (*E* + *S*) partially cut open to show better the esophagogastric junction and normal esophageal mucosa.

DISCUSSION

Despite the absence of an anatomic sphincter in the distal esophagus, the initial resistance to reflux seen at times in the surgically created hiatal hernias suggests the presence of a functional sphincter. Unfortunately, it cannot prevent gastroesophageal reflux from increased abdominal pressure by itself. Maintaining the gastroesophageal angle alone does not necessarily prevent reflux. When, in addition, the normal distal esophagus remains properly buttressed by the crural tunnel and liver, competence results. When the cardioesophageal junction is above the diaphragm, however, a reconstructed angle does not prevent reflux unless the opposite right esophageal border is positioned against a nonyielding surface.

The distal esophagus can be placed in a tunnel made from the fundus or from a plastic sling after the esophagogastric angle is reconstructed. In addition to stabilizing the right esophageal border and creating an oblique angle of entry, the esophagus is effectively closed as the fundus distends. The tunnel also appears to function as an effective one-way valve when the stomach is almost empty. When the tunnel is properly constructed, extending over the distal esophagus to the lesser curvature of the stomach, squamous mucosa should not be exposed to gastric secretions. In this phase of the study the animals have not been subjected to the effects of chronic gastric hypersecretion using histamine in beeswax.

The plastic sling tunnel may have several advantages over the fundus tunnel. The sling tunnel may be made somewhat looser, and there is no concern about the amount of fundus available for making a tunnel. The knotted fiber junctions of the plastic net permit it to be cut to any shape without unraveling. It is easily sterilized by routine autoclaving. The net remains pliable and becomes intimately incorporated with the stomach and esophagus without excess scar formation. Because of the tendency for nylon to lose tensile strength in the body,[1, 18] we now prefer to use a similar knotted net made from Dacron or Teflon.

This principle of re-creating the esophagogastric angle and establishing an oblique channel for the distal esophagus is offered as a simple method for maintaining gastroesophageal continuity without reflux. It should be suitable for the frail poor-risk patient. The procedure may also have application when the cardioesophageal junction is below the diaphragm, but the crura are lax, attenuated, or absent. The inability to vomit with apomorphine stimulation has caused us some concern. Perhaps this might be a less serious objection in the human who can avoid dietary indiscretions and seek relief through a decompressing nasogastric tube if gastric distention occurs. A concomitant pyloroplasty or gastric drainage procedure should be done when the valve is constructed whether or not the vagus nerves are disturbed. Because the 2 dogs with a substituted nylon crus were able to vomit with apomorphine, although otherwise competent, we are now studying the possibilities of maintaining the esophagus against the vertebral area above the diaphragm by direct suturing and the plastic crural sling principle.

SUMMARY

1. A simple method of making a one-way cardioesophageal valve independent of the diaphragm is described. This mechanism permits normal swallowing but prevents gastric reflux into the esophagus.

2. Pertinent studies on human stomach and esophagus specimens are reported. The results using various modifications of the principle in a series of dogs are described.

3. Possible applications of this valve and its modifications in preventing gastroesophageal reflux and esophagitis are discussed.

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Discussion

DR. WILLIAM A. BARNES (New York, N. Y.).—The surgical approach to the correction or treatment of esophagitis falls into 2 main categories. There are those operations the aim of which is to reduce the peptic activity, the acidity, and the volume of the gastric contents so that, should regurgitation occur in the esophagus, these effects would be minimized. The second group of operations are those devised to prevent gastroesophageal reflux. This is the group being dealt with.

The valve mechanism described is certainly ingenious, and wider experience with it may indicate its true place in the surgical armamentarium. At Cornell, Dr. Frank Raidle and I have been interested during the past several years in similar studies, and I would like to show some of the attempts we have made.

(Slide.) This is a stomach in which the esophageal gastric junction has been resected and a tunnel is formed within the wall of the stomach rather than using infolding or overfolding of the entire thickness of the wall. This tunnel is easily raised between the muscular layer and the submucosa, and it may be placed in any portion of the stomach.

(Slide.) This slide shows the esophagus being pulled through this tunnel and the final slide is a cut section showing the position of this esophageal tube. (Slide.) Here, as has been indicated in the previous presentation, there is an outer wall that prevents the bulge that would occur if the cardia were merely sutured along the side of the esophagus. With this procedure, food and barium pass through freely without regurgitation, even with the tests previously described.

There is, however, one interesting change. We have resected the esophagus and, at the point where the esophageal mucosa meets the gastric mucosa, in those animals where this tunnel was formed in the fundus of the stomach, many of them developed localized esophagitis, even though reflux did not occur. In the animals in which this was done in the antrum, however—thus far, in a relatively small group—no esophagitis or ulceration has occurred.

I would like to ask the authors whether or not they have resected any of these dogs and observed that point of the junction of the esophageal mucosa and the gastric mucosa? Certainly, more studies along the lines suggested by these interesting experiments are in order in an attempt to further solve the problem of esophagitis.

DR. W. E. NEVILLE (Cleveland, Ohio).—I would like to discuss the paper in relation to some work that Dr. Clowes and I have been interested in over a period of years, and that is the interposition of the segment of the transverse colon between the lower esophagus and the stomach in the prevention of esophagitis.

In order to evaluate the procedure, we took this problem to the laboratory. (Slide.) This is a specimen of an esophagus, transplanted colon, and stomach in a dog sacrificed at the end of 7 months. There is no evidence of esophagitis, colitis, or gastritis.

(Slide.) This is a specimen removed from an individual that died as a result of cancer at the end of 2½ years. This is the esophagus. This is the transplanted colon. There is no evidence of esophagitis or colitis.

(Slide.) This is an x-ray. On your right, you can see a stricture of the lower end of the esophagus in an 8-year-old child that had swallowed muriatic acid. This was resected with a segment of colon interposed between the esophagus above and the stomach below. This child is essentially a normal individual.

(Slide.) This is a photomicrograph of a segment of the esophagus 1 cm. above the area of the transplanted colon, showing no evidence of esophagitis.

DR. ORVILLE FRANK GRIMES (San Francisco, Calif.).—The problem of esophagitis following the destruction or removal of the cardioesophageal junction still is quite a problem. But the problem of esophagitis following the surgical removal of the cardioesophageal junction or its destruction as a complication of esophageal hernia has been investigated from several approaches in our laboratory.

Recently, working with Dr. Najarian, I performed a series of experimental procedures wherein a naturally occurring intestinal valve was substituted for the excised or pathologically destroyed cardioesophageal sphincter mechanism. This study evaluated the protection afforded the esophagus by the placement of an ileocolic valve between the esophagus and the stomach and the effect of acid peptic digestion on the interposed segments.

In a series of 24 dogs, the segment of ileum varying from 20 to 24 cm. and a short segment of cecum and ascending colon suspended upon the ileocolic arteries were brought up through a rent in the transverse mesocolon behind the stomach, and were used to replace the segment of lower esophagus, cardioesophageal junction, and upper stomach excised by this particular procedure.

Studies in this group of dogs in the postoperative period while the dogs were still alive showed by x-ray that significant reflux into the esophagus was prevented and, at autopsy pressure studies within the stomach showed that some 50 cm. H_2O or more was necessary to produce significant regurgitation.

Daily usage of histamine evaluated the protection afforded the esophagus by this procedure. To show this specimen, here is the duodenum and, then going backward, the stomach area here, the anastomosis of the stomach and the short segment of colon; here, the ileocolic valve, the ileum, and, above, the esophagus.

Now, going the other way, in these experiments, wherein daily doses of histamine were used, it can be seen that the ileum proximal to the ileocecal valve remains free from ileitis and the esophagus likewise remains free. The short segments of colon did have various degrees of inflammation in response to the histamine stimulation and, almost routinely, there would have been gastritis. As you can see from the arrow points here, ulceration, sometimes fatal, occurred in the duodenum.

The next slide is another such specimen. Proximal to the ileocolic valve, the ileum remains free from significant esophagitis, as does the esophagus in its lowermost portion.

The next slide will show one of the human patients operated upon in this way. This was taken at the operating table and I can show you here only that here the esophagus is anastomosed to the ileum, which progresses in this fashion to the ileocolic valve here. The cecum and the appendix have been excised. The anastomosis between the ascending colon and the stomach is in this region. This is merely a sort of reverse Hoffmeister on the greater curvature to approximate more the caliber of the stomach to the colon. We believe that, with this limited clinical experience, this procedure has a certain value in isolated cases.

DR. ADLER.—We, too, were able to create tunnels, submucosal tunnels in the stomach, and Wooler has reported the use of this procedure in 3 human cases (Thorax **11**: 275, 1956). But we were disturbed by the disproportion between the size of the esophagus and the stomach walls so we adopted these methods.

The other 2 discussants mentioned interposed colon and ileocecal valves; those may well be excellent. One of the reasons we were attracted to this method was the fact that it is a rela ively simple thing to do and certainly should be suitable for frail, elderly patients.

(Slide.) This shows the esophageal gastric junction in one of these tunnels at 6 months. Here it is opened with a probe. The stomach has been opened along the greater curvature. The esophagus is open and here you see the probe poking through the tunnel part way open with a clean esophageal gastric junction.

In answer to what Dr. Barnes said, the important thing is if the tunnel is made it must extend to the lesser curvature. In other words, when the tunnel is formed and when this is closed, there can be no squamous mucosa exposed to gastric juice. Otherwise, ulceration may occur.

REGIONAL RENAL HYPOTHERMIA

PAUL STUEBER, M.D. (BY INVITATION), STEVEN KOVACS, M.D. (BY INVITATION), SIMON KOLETSKY, M.D. (BY INVITATION), AND LESTER PERSKY, M.D. (BY INVITATION), CLEVELAND, OHIO

(From the Department of Surgery, Urological and Anaesthesiology Services, and the Institute of Pathology, Western Reserve University and the University Hospitals)

THE kidney, by virtue of its single artery and vein, has been the subject of extensive investigation involving vascular occlusion or constriction[4] and even organ replacement.[2] As early as 1880 Litten[8] demonstrated in rabbits that the renal pedicle could be occluded for one hour before damage was evident upon microscopic examination. Later, Van Slyke,[16] in studying dogs upon which unilateral nephrectomy had been previously carried out, found that mortality increased to 100 per cent with a period of 6 hours of pedicle clamping. Koletsky[7] has made parallel observations in the rat where a somewhat shorter period of anoxia is tolerated. Recent advances in vascular surgery have permitted the separation of the human kidney from its blood supply for varying periods of time in the excision of the aorta for occlusive disease and aneurysm.[3] It has been possible similarly to do a segmental resection and replacement of a portion of the renal artery with a transient period of anoxia.[11]

Attempts have recently been made to study the renal changes occurring as a result of the periods of ischemia attending these replacement procedures. Moyer and his associates[10] showed that there was moderate-to-severe renal damage in the dog when the renal artery or the renal artery and the aorta were occluded for periods of 1 to 3 hours. They subsequently observed that generalized hypothermia significantly reduced this renal damage. In an effort to obviate the known cardiac and thermoregulatory disorders attending generalized hypothermia,[15] we thought it would be of interest to employ regional renal hypothermia in order to study the effects of such local cooling on animal survival, renal morphology, and renal function as indicated by blood urea nitrogen concentrations following a period of 6 hours of pedicle clamping.

PROCEDURE

Utilizing intravenous sodium pentobarbital anesthesia in healthy mongrel dogs, we performed right nephrectomies 2 weeks prior to the experimental period. Subsequently in all animals, the left kidney was exposed through the flank and completely freed of the perirenal fat. All capsular vessels were ligated, and the hilar vessels and ureter were similarly dissected free from overlying fat and connective tissue. In the majority of instances, 1 ml. of heparin diluted with 4 ml. of saline was injected intra-arterially prior to clamping the blood supply (Fig. 1). The clamp was left in place for 6 hours during which time observations were made regularly on renal, rectal, and room temperatures.

This work was supported in part by the U. S. Public Health Service, Grant No. H3682.

Read at the Annual Meeting of the Society of University Surgeons, Boston, Mass., Feb. 13-15, 1958.

Each animal received an intravenous infusion of 500 ml. of 5 per cent dextrose and water. At the end of the occlusive period, the clamp was removed, the kidney was returned to the renal fossa, and the incision was closed. The blood urea nitrogen and urinary output were followed during the subsequent course of the animal. Survivors were sacrificed after 6 to 10 weeks.

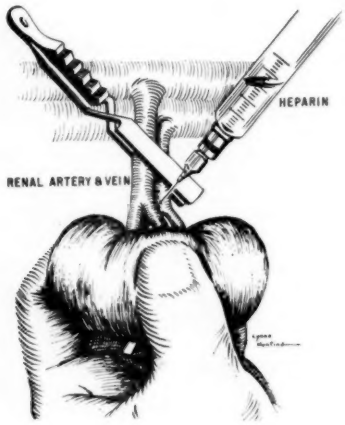

Fig. 1.

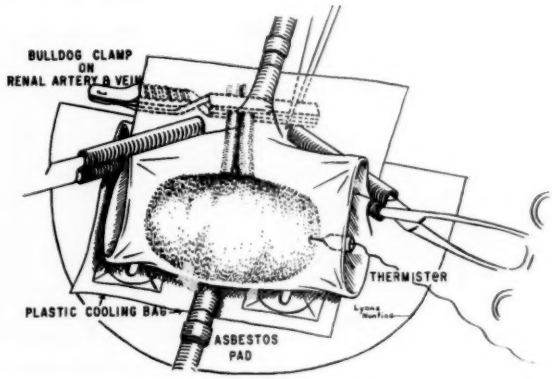

Fig. 2.

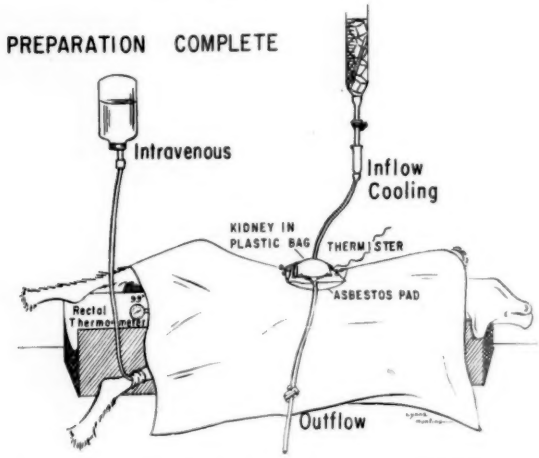

Fig. 3.

Fig. 1.—Injection of heparin into renal artery prior to clamping of the renal pedicle.

Fig. 2.—Kidney surrounded by plastic water jacket and asbestos pad in place at onset of cooling.

Fig. 3.—Preparation completed with continuous circulation of cooling solution through perirenal jacket.

There were 4 series of animals. In the control group of 10 dogs, the kidney was left at room temperature for the entire period. In 3 experimental groups (10 animals in each group), the kidney was cooled during the anoxic period by a plastic jacket. The cooled kidney was insulated from the animal by the use of an asbestos pad (Fig. 2). By means of a continuous flow of water (Fig. 3) cooled to varying degrees by an admixture of ice and salt, it was possible to control the range of cooling, and we arbitrarily studied 3 hypothermic groups: 20° to 25° C., 10° to 15° C., and 0° to 5° C.

RESULTS

Results in terms of percentage of survival are depicted in Fig. 4. There was a 100 per cent mortality in the control group, with a progressive increase in survival in the experimental groups until 100 per cent survival was achieved in the group cooled to 0° to 5° C. Typical blood urea nitrogen levels for each group are shown in Fig. 5. It can be seen that in the control group the blood urea nitrogen showed a rapid elevation which usually continued to rise until death. In the survivors of the group with renal cooling to 20° to 25° C., there was also a rapid rise which usually declined slowly after 4 days. In the group cooled to 10° to 15° C., the rise was less abrupt, the peak was lower, and normal levels were attained after 2 weeks. In the animals cooled to 0° to 5° C., there was usually very little change from the previous group or else no deviation from the normal.

The control animals were autopsied at the time of death which occurred in the majority of instances within one week of cooling. The kidneys from these animals presented a bulging cortex with an indistinct architectural pattern. In the experimental animals which did not survive, the kidney was usually pale throughout, only slightly enlarged or normal in size and gross appearance. Occasionally in these nonsurvivors small, recent cortical infarcts were present.

Microscopically, in the animals that died, the principal lesion was one of coagulation necrosis of the proximal convoluted tubules. There was desquamation of the lining cells, loss of nuclei, and blockage of lumens by coarse acidophilic debris. The glomeruli, the loops of Henle, and the distal convoluted tubules were essentially intact.

In the region of the necrotic tubules, there were focal interstitial collections of lymphocytes. Sites of calcific deposit were noted in the areas of necrosis. There were numerous, homogenous, pink-staining casts in the collecting tubules.

The tubular necrosis was widespread and massive (Fig. 6) in the control series of animals. Evidence of repair in these proximal tubules, including the descending or spiral segment, was usually slight. The necrotic lesion in the cooled animals that failed to survive was similar but less severe and extensive. Usually the damage was slight and consisted of focal degeneration or necrosis of the proximal tubules. Repair was rapid, and after 1 to 2 weeks new cells frequently lined the dilated tubules. Focal calcific deposit persisted (Fig. 7). Dogs sacrificed after 8 to 10 weeks showed practically normal kidneys (Fig. 8).

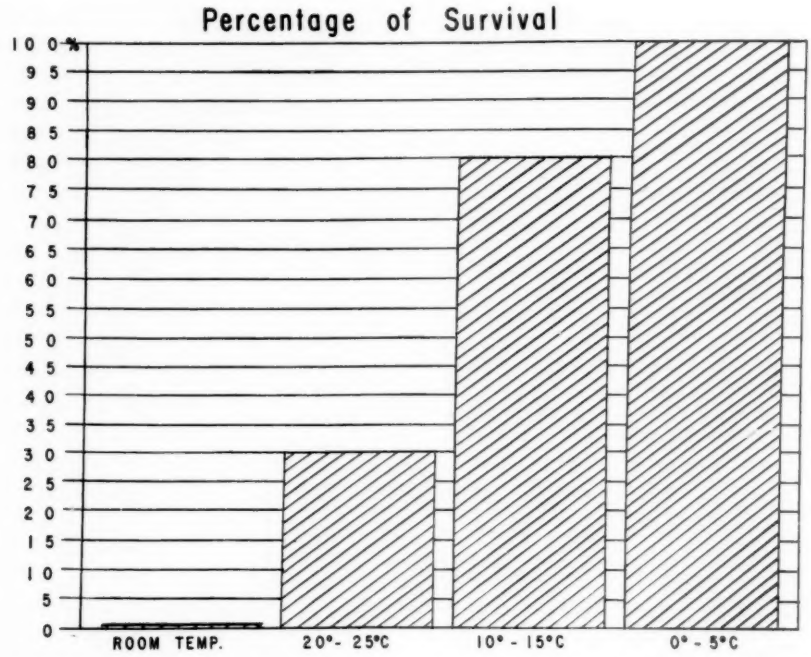

Fig. 4.—Percentage of survival found in various groups. The control group of animals at room temperature had 100 per cent mortality; at 0° to 5° C. of cooling there was 100 per cent survival.

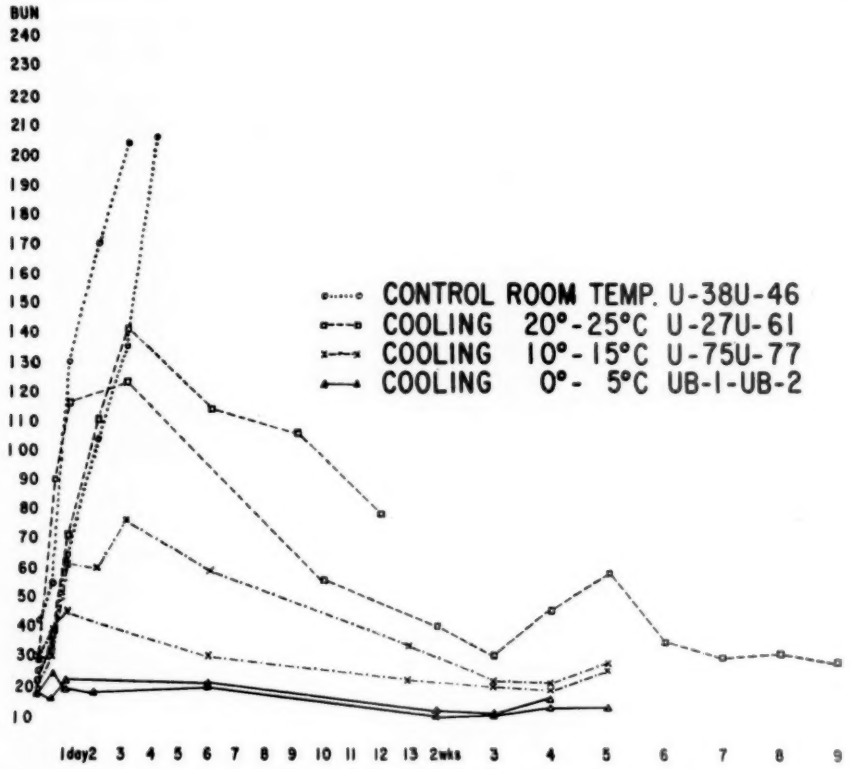

Fig. 5.—Typical curves for blood urea nitrogen concentrations following renal pedicle occlusion. Only slight deviation was noted in the group cooled to 0° to 5° C.

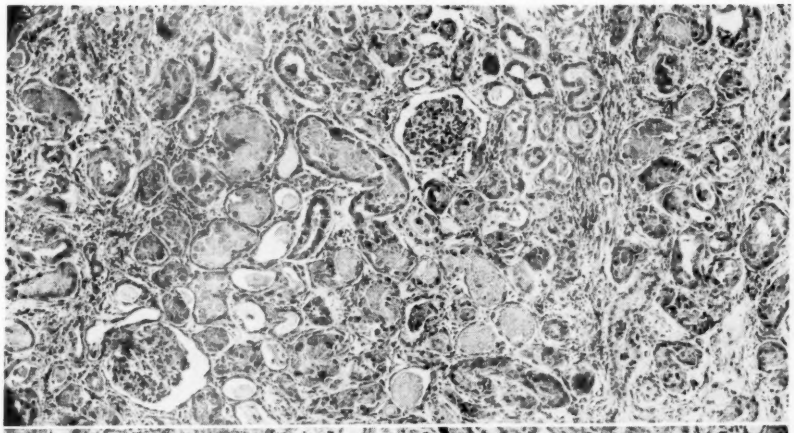

Fig. 6.

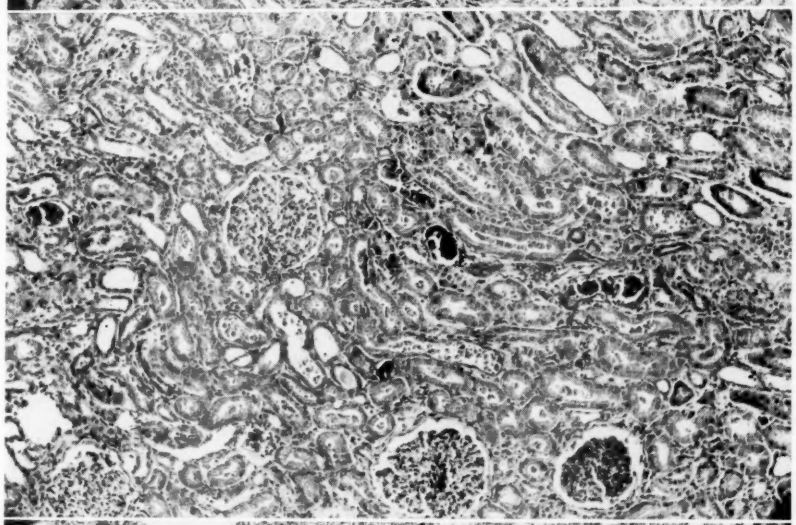

Fig. 7.

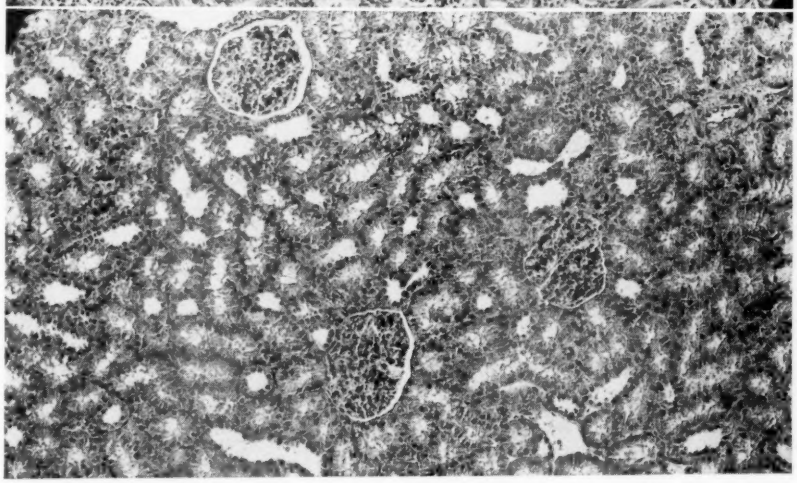

Fig. 8.

Fig. 6.—Kidney section from animal that failed to survive. Widespread necrosis is noted of the epithelium of the proximal convoluted tubule.

Fig. 7.—Section of kidney showing recovery with focal areas of calcification.

Fig. 8.—Kidney of surviving animal which shows little change from normal.

DISCUSSION

These observations parallel those of Schloerb and associates[14] who studied local kidney cooling at 0° to 5° C. for periods varying from 1 to 12 hours. They similarly had 100 per cent survival in their animals. However, in a group in which in vitro cooling was attempted, only indifferent success was reported. Bogardus and Schlosser[1] also studied local cooling and found that a protective effect was afforded by cooling to temperatures of 16° to 30° C. Goodwin,[5] in reviewing their report, described an initial attempt at local cooling in segmental resection of the kidney in humans.

Mitchell and Woodruff[9] of New Zealand have also applied renal hypothermia to renal autografts in sheep with success attending transplants carried out at 10° to 20° C. and a 2-hour period of ischemia. When cooling was not employed, a far greater incidence of failure attended their efforts. Just as renal physiologists, surgeons, and urologists have been stimulated to attempt local cooling of the kidney, similar efforts have been directed toward studying regional hypothermia of the brain and liver.[6] The accessibility of the kidney, however, and the uniqueness of its blood supply make successful clinical application of local cooling a distinct possibility. With renal viability provided during prolonged clamping of the renal pedicle for periods of longer than 10 to 15 minutes as originally suggested by Prather,[12] segmental resection, extensive nephrolithotomy, and a bolder attack in general upon many correctible or reversible renal lesions may be undertaken.

There are other interesting facets of these preliminary studies. In the rat, hypertension has been seen to develop in the long-term follow-up of animals with varying periods of renal ischemia.[7] We hope to determine if similar results will be seen in dogs followed for longer periods of time prior to extensive clinical application. Vermeulen[17] noted parallel tubular lesions and calcification in animals subjected to ischemia without hypothermia. He also felt that with healing and time these areas of calcification tended to lessen and has conjectured about a possible tool for the study of renal calculi.[17]

In half of the animals cooled to 0° to 5° C., no heparin was employed and survival was not affected. An interesting avenue of investigation may be afforded by a study of the intravascular changes and the clotting mechanisms following regional ischemia and hypothermia. In an early pilot study we attempted renal chilling without pedicle clamping. Slight generalized body cooling accompanied a somewhat greater depression of renal temperature after several hours. Perhaps this technique might afford a means of achieving a combination of local and generalized hypothermia while avoiding the risks of severe generalized body cooling.

In summary, it can be stated that in the dog regional renal hypothermia affords definite protection from lethal ischemia. Successful clinical application seems a distinct likelihood and numerous opportunities exist for further study and investigation of renal physiology, hypertension, coagulation mechanisms, and organ transplantation.

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(For discussion, see page 88.)

TOTAL BODY COOLING IN CRITICALLY ILL FEBRILE PATIENTS

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THIS study was undertaken to evaluate the effectiveness of total body cooling on critically ill febrile patients.

The treatment is an ancient one and we do not know who first used total body cooling in febrile patients. Ice baths and alcohol sponging were the usual methods, but with the development of special refrigeration equipment and a means of preventing shivering the full potentialities of total body cooling are becoming apparent. Successful use of total body cooling on such patients has been reported, in recent years, by Lewis and associates,[2, 3] Vaghelyi and Magy,[4] and Jesati[1]; however, critical evaluation of the procedure with control studies has been lacking.

The total effects of body cooling on the course of febrile patients have not been reported. We do not know, for example, whether or not cooling alters the relationship between the host and the disease. Until these factors are known, control studies on survival, such as the one reported here, are valuable.

METHODS

Selection of Patients.—The criteria for selection of the patients were (a) an oral temperature of at least 40° C. (104° F.), (b) a pulse rate of at least 120 per minute, and (c) a respiratory rate no less than 24 per minute. All possible treatment for cause of the febrile condition had been exhausted and a fatal outcome was thought to be likely. Once the patient was accepted for the study the entire care of the patient was entrusted to the staff in charge of this study.

Each patient was placed in either a cooled group or a control group, selection being made by use of a series of random numbers, supplied by the University of Minnesota Biostatistics Department. A total of 13 patients comprised the control group and an equal number formed the cooled group.

These patients varied in age from 4 years to 81 years with a median age of 55.3 years. They suffered a wide variety of illnesses and major complications (Table I).

Method of Cooling.—All patients were cooled by means of the refrigerating machine manufactured by the Thermo-O-Rite Products Corporation of Buffalo, New York. The rubber blanket to this apparatus, through which fluid circulates, was placed on the mattress of the bed and covered with a single cotton blanket. The patient was placed on this blanket and left exposed to the room air except for a loin cloth. In some large obese patients, a second rubber cooling blanket was placed on top of the patient's body to facilitate the induction of the cooling. However, once the desired body temperature was obtained this second cooling blanket was discontinued.

Supported in part by Research Grant No. H-2879 of the United States Public Health Service.

Read at the Annual Meeting of the Society of University Surgeons, Boston, Mass., Feb. 13-15, 1958.

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TABLE I. SURFACE COOLING OF FEBRILE PATIENTS

TYPE OF CASE	NUMBER	
	COOLED	CONTROL
Complications following general and thoracic surgery	5	6
Complications following head injuries and neurosurgery	4	3
Complications following urologic surgery	1	0
Burns (25 per cent body surface)	0	1
Medical problems	3	3
Total	13	13

Intravenous chlorpromazine was used for sedation in amounts sufficient to prevent shivering. On induction of the cooling process an average of 100 to 150 mg. of chlorpromazine was given intravenously. Subsequently, 50 to 100 mg. was administered intravenously every 2 to 4 hours. Sodium phenobarbital was also given intravenously in amounts of 60 to 70 mg. alternating with the chlorpromazine. This helped to diminish apprehension. The maximum amount of chlorpromazine used to prevent shivering was 1,200 mg. daily for 3 days, but most cases required only 300 mg. chlorpromazine during the first 24 hours. No side effects or toxic phenomena were noted.

The patients were cooled until a rectal temperature of 35° to 36° C. (95° to 97° F.) was reached. This body temperature was easily maintained with a minimum of medication for any desired period of time. After 36 to 48 hours of total body cooling, the patient was allowed to rewarm gradually. Cooling was restarted if his rectal temperature rose above 38° C. (100° F.). The longest duration of total body cooling was 42 days. This patient survived.

CRITERIA FOR EVALUATION

The results are tabulated under 3 headings.

A. Survival.—A patient that lived 30 days or more after institution of total body cooling was considered as having survived for purposes of this study.

B. Clinical Improvement.—This was a subjective criterion based upon physical examinations and the clinical appearance of the patient. It could not be verified by laboratory studies. Unfortunately many of these patients had underlying pathology that was not amenable to any definitive treatment and final cure. Therefore, the duration of life, short of a survival, with the following standards, substantiated this criterion: (1) a decrease in the pulse rate to less than 100 per minute, (2) a decrease in the respiratory rate to 24 per minute or less, and (3) stabilization of the blood pressure at normal levels.

C. No Clinical Improvement.—Patients not meeting these standards of benfit were considered to have received no clinical improvement.

RESULTS

The results obtained are tabulated in Tables II, III, and IV. The only survivors occurred in the cooled group. Definite clinical improvement was noted in the rest of the cooled group except for 2 patients who expired before the cooling process could be instituted.

There were no survivors in the control group. Clinical improvement was noted in only 3 patients. Ten had no improvement.

The benefits of total body cooling for patients who had complications following general and thoracic surgery are illustrated in Table II. Benefits derived by patients with head injuries and other neurosurgical complications are listed in Table III. We have the impression that the neurosurgical patients received the greatest benefit from total body cooling. The results of cooling nonsurgical patients are recorded in Table IV.

TABLE II. TOTAL BODY COOLING FOR COMPLICATIONS FOLLOWING GENERAL OR THORACIC SURGERY

RESULT	NUMBER OF PATIENTS	
	COOLED	CONTROL
Survived	1	0
Improved	3	1
No improvement	1	5
Total	5	6

TABLE III. TOTAL BODY COOLING FOR HEAD INJURIES AND NEUROSURGICAL COMPLICATIONS

RESULT	NUMBER OF PATIENTS	
	COOLED	CONTROL
Survived	2	0
Improved	2	1
No improvement	0	2
Total	4	3

TABLE IV. TOTAL BODY COOLING FOR NONSURGICAL COMPLICATIONS

RESULT	NUMBER OF PATIENTS	
	COOLED	CONTROL
Survived	0	0
Improved	2	1
No improvement	1	2
Total	3	3

DISCUSSION

The use of total body cooling in critically ill febrile patients is not directed at the underlying disease process but at the hyperpyrexia. The rationale of its use is acceptable so long as cooling does not decrease the body's ability to fight the disease and so long as cooling does not increase the virulence of the disease process. The validity of this rationale may not be settled by this clinical study, but the general response of the febrile patient to cooling appeared to be favorable.

The pulse and respiratory rates, as illustrated in Fig. 1, decreased in direct relationship to the drop in rectal temperature. These changes signify a decrease in circulatory and respiratory work. Tidal air volume increases and physical rest is possible. Improvement in the appearance of the patient is often dramatic.

Daily laboratory studies performed in both the cooled and control groups included complete blood counts, serum chloride, serum sodium, serum potassium, and blood carbon dioxide combining power. No significant changes were noted in any of these determinations. Scattered bleeding and clotting time determinations in both groups were all within normal limits.

Cooling to the levels used in this study apparently did not diminish the body's ability to respond to acute infections. This was illustrated in one of the survivors (P. T.). This 6-year-old white boy had sustained a depressed skull fracture with a laceration of the cerebrum and a fractured right femur.

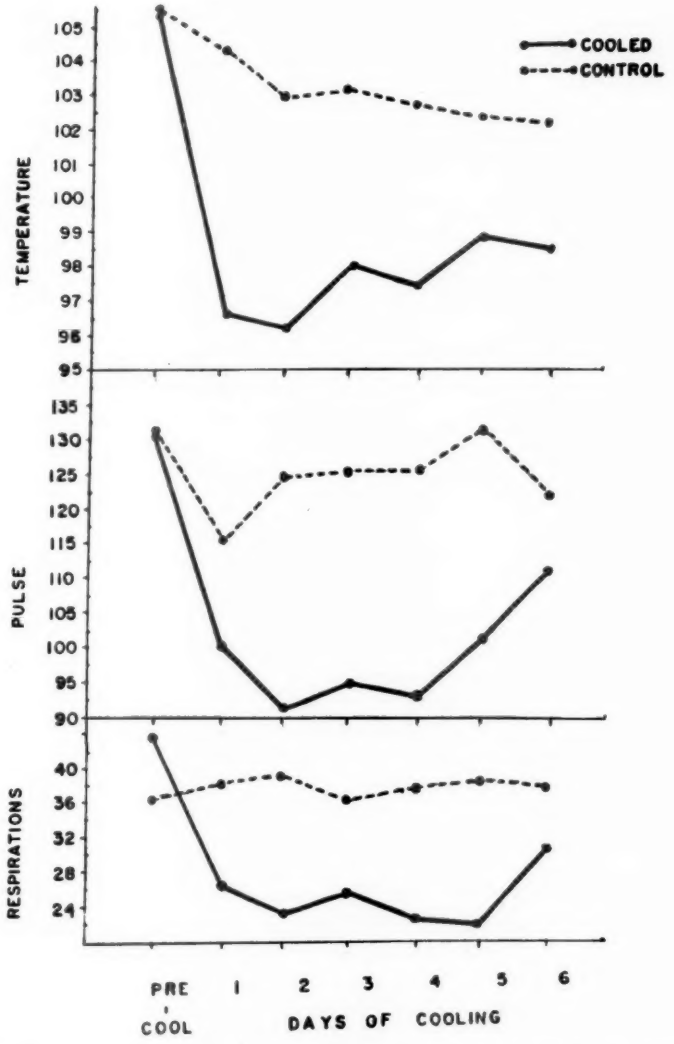

Fig. 1.—Mean temperature, pulse, and respiration rates of all patients in control and cooled groups.

The skull fracture was elevated shortly after admission but his temperature rose to 41° C. (106° F.) and his general condition deteriorated. Two days after admission he was placed in this study. After 14 days of cooling the patient developed a purulent pericarditis that required multiple aspirations and finally an open drainage of the pericardial sac. During this acute infectious exacerbation the patient's leukocytes increased from 16,000 to 104,000. After the surgical drainage was accomplished, the leukocytes decreased to 14,000.

Total body cooling of critically ill febrile patients has been an effective adjunct to therapy. No clearly deleterious effects have been noted and this study favors its further use.

SUMMARY

1. Twenty-six critically ill febrile patients were divided into 2 groups by random sampling. One group was subjected to total body cooling to the level of 35° to 36° C. and the other remained as a control group.

2. Three survivors were obtained in the cooled group while there were no survivors among the control group.

3. Clinical improvement occurred in 8 of the cooled group but in only 3 of the control group.

4. No significant changes were noted in the hemoglobin, serum chlorides, serum potassium, serum sodium, or blood carbon dioxide combining power in either group.

5. Total body cooling to 35° to 36° C. does not prevent blood leukocyte mobilization.

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DISCUSSION

(Papers by Stueber, Kovaks, Koletsky, and Persky; Reeves and Lewis)

DR. HENRY PAGE ROYSTER (Philadelphia, Pa.).—I would like to report one experience that happened to me 22 years ago as an intern. A worker who washed glassware in a Rohm & Haas chemical branch office in Philadelphia was brought into the hospital in heat shock. This was June 30 and was a very warm day. Her temperature was 109° F. rectally. Of course, we had none of the advantages of cooling apparatuses we have today. We started ice water going through every aperture. First was an ice water enema, then ice water in the stomach, ice water in the vagina, ice water intravenous at 42° C.—the temperature of the blanket; we laid the patient on a rubber sheet and put ice all over her, that is, buried her as you would a fish. This was at five o'clock in the afternoon and, at seven o'clock, her temperature had dropped 2 degrees. The patient recovered within a few hours, and her state was then returned to normal. She went back to work.

DR. C. BARBER MUELLER (Syracuse, N. Y.).—We have begun using cooling for critically ill patients at Syracuse. The longest we have kept patients cooled was 3 weeks. That was with the thermocouple in the anus. At present, we have only ice bags, which makes cooling a prodigious job requiring full-time nursing care.

We observed then that these people will subsist with a urinary output of about 200 ml. per day without any concomitant rise in the nonprotein nitrogen. It is possible to titrate a level of consciousness, frequently depending of course on the cause of their serious illness. A couple of our patients have been brought in and out of consciousness by regulating their temperature from 90° to 92° F.

We have likewise noticed that, as you warmed them up, very frequently the respiratory ability would be so inadequate that, as they reached about 92° F., they would become cyanotic and, if one cooled them back down to about 90° F., they would become pink again. The whole series of metabolisms seems to be slowed down to the point where you must be a little careful in fluid therapy.

DR. JACK MATHEWS FARRIS (Los Angeles, Calif.).—The general rule of cooling in surgical patients has been emphasized for almost a decade, particularly by our cardiovascular colleagues, and I do not wish to take part in the debate whether or not the hypothermia is superior to the pump-oxygenator techniques. I feel that many of us who perhaps have more interest in general surgical problems have been remiss in adapting hypothermic or like-hypothermic techniques, both in the preoperative and the postoperative care of febrile patients.

We have been interested for about 3 years in cooling critically ill patients and also have had the experience of salvaging a girl with a hopeless, previously considered inoperable gangrenous intestinal obstruction with generalized peritonitis, a temperature of 107° F., and imperceptible pulse and blood pressure. With cooling techniques like hypothermia, she made a recovery which, although obviously it is not open to scientific control, would have been unlikely without this technique.

We have been extremely impressed with the urinary outputs in critically ill patients. We operated upon a man with a combined abdominal peritoneal resection with emphysema and respiratory acidosis. After this operation, he excreted 100 c.c. of urine. Ten days later, he was critically ill with an intestinal obstruction and was operated upon under much more precarious conditions and, from this operation, excreted 1,700 c.c. of urine in his first 24-hour period.

We have observed this same phenomenon on a number of occasions. It suggests that the stress reaction to trauma and anesthesia is nullified by hypothermia. It has been our experience that shock may be delayed until 24 or 48 hours later. One must be on the alert for this late complication.

DR. HENRY H. BALCH (Washington, D. C.).—It is very difficult to know, when you are using this sort of technique and getting a good result, whether it is the effect of the cooling or the multiple procedures being done.

In studying peritonitis in rats, we have endeavored to see whether or not we could produce any effect with cooling. We took 2 groups of rats, used a standardized procedure of producing peritonitis which we had used previously, and were able to show that, with cooling to 28° C. and other therapy, survival could be prolonged for several hours by cooling. We also were able to show by using a bactericidal antibiotic such as streptomycin that survival could be prolonged just as well without cooling. It did not appear in these studies that cooling presented any additional advantage.

We were able to predict the depth of survival quite well doing quantitative bacterial counts from the peritoneal exudate. There is a paradox here. In infections, apparently the rate of bacterial growth presumably is slowed down with cooling. You also may interfere with the efficacy of the antibacterial defense with cooling. If one is to cool patients for bacterial infections, he would have to be very careful to cover them with antibacterial therapy.

DR. DONALD D. MATSON (Boston, Mass.).—Dr. Lewis did not directly malign the neurosurgeons, but I think there was a certain implication perhaps. One of the difficulties in the observations regarding cooling in these critically ill patients has been the lack of any scientific control of any individual patient. Perhaps the closest we have come to that has been in observation of 2 or 3 patients with severe head injuries who have been cooled and in whom one could regulate or measure at least clinically quite accurately certain observations during their cooling and rewarming.

I could cite one particular patient, a child of about 12 years with a severe closed head injury with the common signs of severe brain stem contusion, that is, decerebrate

rigidity, dilated pupils, failure to react to pain, irregular Cheyne-Stokes type of respiration, and uncontrolled hyperthermia. When she was cooled to approximately 90° to 92° F., her pupils came down, she reacted to pain, she lost her decerebrate rigidity, and so on. At an interval of perhaps 3 or 4 days, she was allowed to rewarm and, when she reached 95° or 96° F., the signs all reappeared. In short, we tried this 2 or 3 times and it was about 2 to 2½ weeks before she could finally be warmed to a reasonable state without these signs reappearing and went on to a good recovery.

DR. LEWIS (Closing).—In the patients I described, we did not use hypothermia. They were just lowered to a normal temperature. The lowest temperature was about 96° F. None of my data has any evidence for or against the idea that cooling these ill patients to hypothermic levels would help even more. I do not know whether or not it would.

We have noticed this phenomenon of a patient being in a satisfactory condition at a temperature of 96° F. and deteriorating immediately when the temperature crept up to 99° F.

One patient has been on the blanket for 42 days with this system. Most patients have only needed the cooling for 1 to 3 days.

ENDOCRINE INFLUENCES OF THE PANCREAS ON GASTRIC SECRETION

I. The Effects of Glucagon and Alloxan on Heidenhain Pouch Secretion

Henry W. Mayo, Jr., M.D., and Daniel M. Enerson, M.D. (By Invitation), Syracuse, N. Y.

(From the Department of Surgery, State University of New York Upstate Medical Center)

PANCREATIC participation in the hormonal control of gastric secretion has been suggested by numerous physiologists and clinicians. In 1955, Zollinger and Ellison[20] reported 2 cases of peptic ulceration of the upper jejunum associated with excessive secretion of acid by the stomach in which multiple surgical procedures failed to prevent recurrent ulcer and in which pancreatic islet cell tumors were ultimately found. The following year, Ellison[4] collected a series of 24 similar cases. In only 2 of these 24 cases were beta cells identified in the neoplasm; both of these patients had developed spontaneous hypoglycemia. Five of these patients had multiple pancreatic adenomas, and 5 had tumors of other endocrine organs.

Zollinger noted that paper electrophoretic analysis of the serum of one of his patients demonstrated the presence of a protein fraction similar to glucagon and that experimental animals injected with this fraction developed transient hyperglycemia. However, these results could not be reproduced on repetition of the study.

These reports stimulated renewed interest in the endocrine interrelationship between pancreas and stomach. Dragstedt[2] noted that peptic ulcers are exceedingly uncommon in pancreatectomized dogs but that such ulcers develop in almost all dogs with pancreatic fistulas. Poth's group[11-13] attempted to evaluate pancreatic influence on the development of peptic ulcer. In 4 pancreatectomized dogs in which diabetes had been controlled with insulin and 4 dogs in which the pancreatic ducts had been ligated, the daily administration of histamine in beeswax resulted in the development of multiple peptic ulcers in the stomach and duodenum as was noted in the histamine control group. Alloxan diabetes was produced in 4 dogs and, following the stabilization of blood sugar concentration by daily insulin therapy, these animals were given histamine in beeswax daily for 10 to 15 days. One animal developed a perforated ulcer, but 3 other animals failed to develop ulceration. The same workers also noted that approximately half of the dogs maintained in a state of hypoglycemia by repeated administration of insulin developed peptic ulceration. Using the same methods, these workers found that the incidence of ulceration did not change following total pancreatectomy.

Read at the Annual Meeting of the Society of University Surgeons, Boston, Mass., Feb. 13-15, 1958.

Poth suspected that the hyperglycemic-glycogenolytic factor in commercial insulin, later called glucagon, had some relationship to ulcer formation. However, he found no difference in the incidence of ulceration following prolonged hypoglycemia produced by the repeated administration of commercial insulin, as compared to prolonged hypoglycemia produced by purified insulin not containing the hyperglycemic factor.

Several recent efforts have been made to determine the relationship, if any, between glucagon and gastric function. Earle and his co-workers[3] found that glucagon administered intravenously to anesthetized dogs consistently lowered the blood pepsinogen levels and that this phenomenon was unchanged following section of the vagi. A decrease in the gastric acidity and the blood pepsinogen levels was also noted in human subjects after the intravenous administration of glucagon. Stunkard, Van Itallie, and Reis[16] thought that glucagon might contribute to the mechanism of satiety, since gastric hunger contractions were promptly abolished in human subjects by the intravenous administration of glucagon and the sensation of hunger was proportionally decreased. Robinson, Harris, Hlad, and Eiseman[14] found that hydrochloric acid output and gastric motility were diminished following the intravenous injection of glucagon. Poth[10] noted stimulation of acid secretion in approximately 80 per cent of patients, usually at about 2 hours and again at about 3½ hours after administration of the glucagon.

Recently Barcena[1] reported that in 2 dogs in which concomitant total pancreatectomy and construction of a Heidenhain pouch was carried out the 24-hour gastric secretory output rose progressively until the time of death 57 and 58 days later, respectively. In 2 other animals, the 24-hour gastric secretory output from Heidenhain pouches was determined both before and after total pancreatectomy; in one of these there was a marked rise after pancreatectomy, but in the other there was no significant change.

The present study was undertaken in an effort to determine whether there might be a hormonal relationship between factors elaborated by the islets of Langerhans of the pancreas and the secretion of hydrochloric acid by the stomach.

METHODS

Heidenhain (denervated) pouches were created in 9 mongrel dogs under intravenous sodium pentobarbital anesthesia. Each pouch was drained with a nylon cannula brought out through a stab wound in the abdominal wall, and to each cannula was attached a bite collar to prevent retraction. Gastric juice from the pouches was collected in rubber balloons attached to the cannulas.

After a rest period of at least 4 weeks, collections of pouch gastric juice were made hourly for 5-hour periods in the fasting state. Such collections were made repeatedly for each animal. The volume of these collections was measured, and the concentration of free hydrochloric acid determined by titrating with 0.1 N sodium hydroxide, using Topfer's reagent as an indicator. In like manner, hourly collections over a 5-hour period were made after the ingestion of a standard 200 Gm. meat meal, and the volume and acidity of each specimen were determined.

Glucagon* was dissolved in a glycine buffer solution, made up in a concentration of 75 millimols per liter with a pH of 9.5. The concentration of glucagon in this solution was 118 mcg. per milliliter. This preparation was preserved in the frozen state, and repeated biologic assays indicated that freezing did not result in any loss of hyperglycemic potency. Assays of the glycine buffer indicated that it had no intrinsic hyperglycemic properties. The quantity of glucagon used (300 to 600 mcg. per dog regardless of size) was many times the amount necessary to produce hyperglycemia, but it was given in this amount in an attempt to evoke a maximum secretory response.

Glucagon was administered intravenously to each of 9 pouch animals in the fasting state repeatedly, drawing blood sugar determinations before and 20 to 30 minutes after each administration. After each injection of glucagon, 5-hour collections of pouch juice were made, and volumes and acidity determined.

After the above studies had been done, alloxan monohydrate was given to each animal intravenously. The amount of this material given to 5 animals was 75 mg. per kilogram of body weight. Three animals received 60 mg. per kilogram of body weight. No attempt was made to control the resulting diabetes with insulin or to maintain hydration or electrolyte balance with intravenous therapy. Periodic examinations of the urine for acetone were made in each animal, and in 3 animals nonprotein nitrogen and chloride determinations were made periodically after alloxan administration. Beginning on the day following the administration of alloxan, daily fasting blood sugar determinations were made for 3 weeks or until the animal expired. Each day, a 5-hour collection of gastric pouch juice was made, either in the fasting state or in response to the stimulus of a 200 Gm. meat meal.

RESULTS

The acid output of the Heidenhain pouch of each dog in the control fasting state was almost negligible but there was a definite secretory response to the stimulus of a 200 Gm. meat meal.

Within 30 minutes following the intravenous administration of glucagon, there was a rise in blood sugar concentration to a value which varied between 24 and 144 per cent above the fasting concentration (Fig. 1). In 6 of the 9 animals, there was a definite fall in the fasting pouch secretion following the administration of glucagon when compared to the control fasting secretions. In one animal there was a slight rise above the fasting rate, in another there was a marked rise, and in one there was no significant change in the average amount of acid secreted after glucagon.

Following the administration of alloxan, the blood sugar concentrations in each animal were elevated to diabetic levels within 48 hours. The hydrochloric acid secreted by the pouch in each animal increased concomitant with or even before the rise in blood sugar concentration. The results of the acid determination on the pouch secretions before and after alloxan are charted in Fig. 2. There was a definite increase in secretion, even in the fasting state, once alloxan diabetes had developed, but this increase was particularly pronounced in response to a 200 Gm. meat meal. Several of the dogs were markedly polydipsic after alloxan. A striking finding was the tremendous increase in hydrochloric acid output with water intake only in comparison to the prealloxan state with ingestion of water only.

*Kindly supplied by Dr. W. R. Kirtley, Eli Lilly & Co., Indianapolis, Ind.

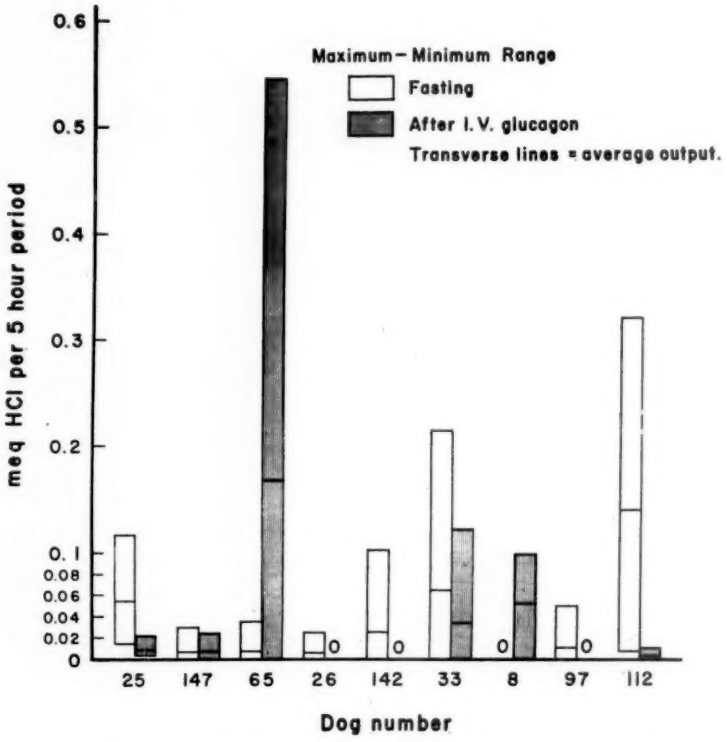

Fig. 1.—Glucagon effect on Heidenhain pouch output.

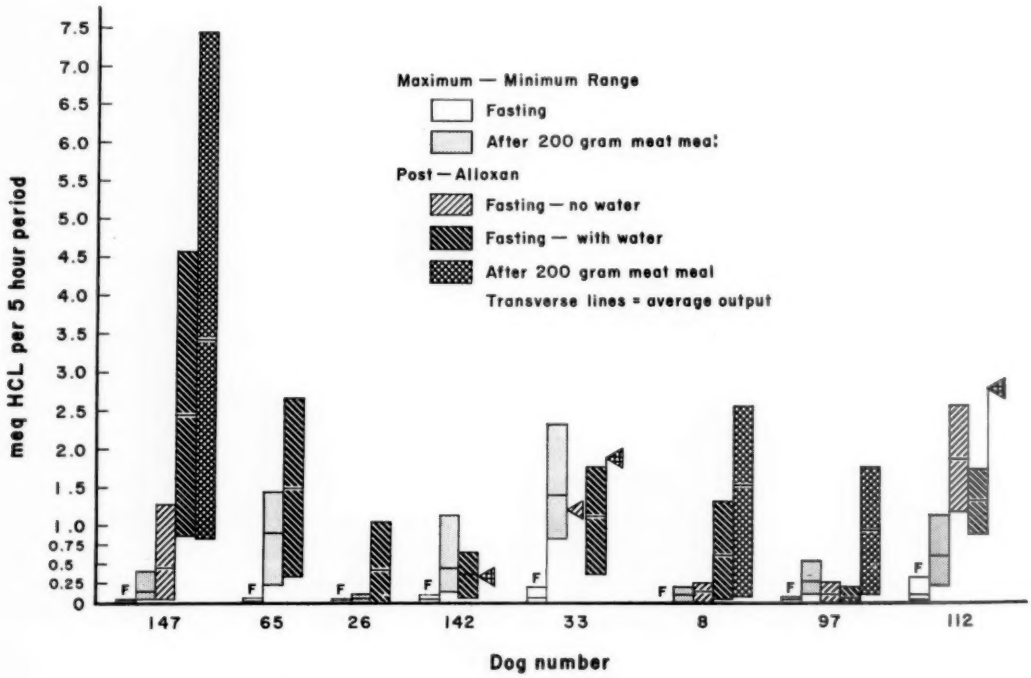

Fig. 2.—Alloxan effect on Heidenhain pouch output.

Occasional examinations of the urine for acetone were consistently negative. Plasma chlorides and nonprotein nitrogen were periodically determined in 3 animals. The chlorides were significantly reduced in 2 of the 3 after alloxan administration, and in the same 2 there was marked elevation of nonprotein nitrogen.

A transitory diabetes was produced in one dog; his blood sugar concentration reached diabetic values (660 mg. per cent) 5 days after the administration of alloxan but then declined steadily, reaching an almost normal value of 140 mg. per cent 2 weeks after the administration of alloxan. As the blood sugar concentration in this animal returned to normal, there was a steady decrease in pouch output, though it was still elevated when compared to pre-alloxan output.

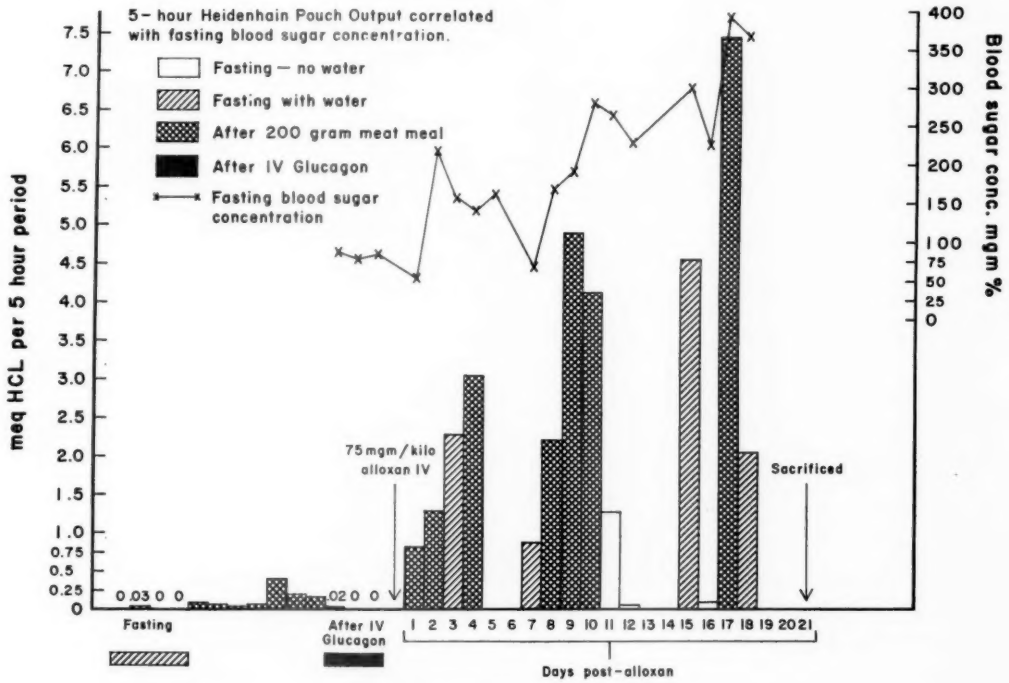
5- hour Heidenhain Pouch Output correlated with fasting blood sugar concentration.
Fasting — no water
Fasting with water
After 200 gram meat meal
After IV Glucagon
Fasting blood sugar concentration
meq HCL per 5 hour period
Blood sugar conc. mgm %
75 mgm/kilo alloxan IV
Sacrificed
Fasting
After IV Glucagon
Days post-alloxan

Fig. 3.—Dog No. 147.

All of the dogs lost weight after the administration of alloxan. The condition of most of them deteriorated, but 3 of these animals survived 3 weeks in fairly good condition after which one was sacrificed and the other 2 were used for other studies. The course of one of these animals is detailed in Fig. 3. The other 5 animals survived 3, 5, 6, 8, and 9 days, respectively, after the administration of alloxan. Dog No. 65 developed an ulcerative lesion at the junction of the gastric mucosa and skin, where a screw from the bite collar had eroded. On the third day after the administration of alloxan, an exsanguinating hemorrhage occurred from this ulcer. Dog. No. 26 died of pneumonia 5 days after alloxan administration. The remaining 3 animals died presumably in uremia; autopsy section of their livers and adrenal glands was not particularly remarkable, but there was a marked degree of necrosis in the proximal convoluted tubules of the kidneys.

DISCUSSION

Glucagon, which presumably is secreted by the alpha cells of the islets of Langerhans, acts to produce release of liver glycogen into the blood stream.[19] Thorogood and Zimmermann[17] showed that the glycosuria is more severe and the insulin requirements are higher in alloxan-treated than in pancreatectomized dogs, but the former are able to live much longer without insulin treatment and fail to develop ketosis and coma. With pancreatectomy following treatment with alloxan, the insulin requirement is less, but the animals quickly pass into coma when not controlled with insulin. They thought for this reason that there was a second endocrine factor (presumably glucagon) acting to increase the blood sugar but to prevent ketosis in the insulin deficient animal.

The results of secretory studies carried out in this study after the administration of glucagon are similar to those of Robinson and his co-workers[14] in human subjects. Certainly, under the conditions of these experiments, in all but 2 instances glucagon did not act as a stimulus to the secretion of acid from the denervated pouch, and there is a suggestion in these results that glucagon may actually inhibit acid secretion.

It is quite unusual to encounter peptic ulcer in association with diabetes in patients. The consistent marked increase in acid secretion following the development of alloxan diabetes was therefore totally unforeseen and quite impressive. The magnitude of this increase in acid secretion varied with each animal. The increase after alloxan was less pronounced in the fasting state, more pronounced if the animals were allowed water, which presumably produced antral stimulation by mechanical distention of the antrum, and most pronounced after the test meat meal.

The dosage of alloxan (75 mg. per kilogram) in 6 animals was certainly sufficient to produce renal damage,[7] and in 2 of the 3 animals that had smaller dosage (60 mg. per kilogram) the nonprotein nitrogen was sufficiently elevated to indicate a marked degree of renal damage. Nevertheless, it is difficult to explain the increase in acid secretion on the basis of renal damage. Madden and associates,[9] in studying the Shay rat, noted that bilateral ligation of the ureters resulted in a decreased volume and acidity of gastric secretion. The same workers indicated that dehydration resulted in a similar decrease in gastric secretion.

There is a possibility that the imbalances created by the administration of alloxan result in a persistent adrenocortical stimulation. Numerous workers[6, 8, 18] have shown that persistent adrenocortical stimulation will result in an increased secretion of hydrochloric acid and pepsin by the stomach. Moreover, in the adrenalectomized animal, substitutive therapy will also result in a similar increase in secretory response to a standard stimulus.[15] This is the usual explanation given for the development of "stress ulcers" or of peptic ulcers developing in a patient on steroid therapy. French and his co-workers,[5] on the basis of studies in monkeys, thought that a secondary fall in pH of the gastric contents of their animals, which occurred about 3 to 4 hours after the administration

of insulin, was explained by stimuli transmitted through the hypothalamus-pituitary-adrenocortical axis. Perhaps some such mechanism comes into play in the alloxan-treated animals in this study. This facet of the problem remains to be studied.

However, an attractive working hypothesis could be formulated to explain this phenomenon on the basis of direct endocrine pancreatic influence. The actual function of the components of the islets of Langerhans are still understood poorly. It is known that glucagon, presumably secreted by the alpha cells, and insulin, presumably secreted by beta cells, are to some extent mutually antagonistic in relation to carbohydrate metabolism. It might seem reasonable, for the purpose of planning future work on the problem, to draw the inference by analogy that there might be a stimulatory and an inhibitory hormone secreted by the pancreas, both of which influence gastric secretion under certain conditions. Thus, a possible explanation of the marked increase in gastric secretion following the development of alloxan diabetes would be that an inhibitory hormone secreted by the beta cells had been abolished by the action of the cytotoxin.

SUMMARY

1. Hydrochloric acid output of Heidenhain pouches in the fasting state over a 5-hour period was compared before and after the intravenous administration of glucagon. In 6 of 9 animals, there was a decrease in acid secretion, while in one there was no change, in one there was a moderate increase, and in one a marked increase.

Under the conditions of these experiments, glucagon does not act as a consistent stimulus to gastric acid secretion, and there is a suggestion that it may inhibit such acid secretion.

2. Alloxan diabetes was produced in 8 dogs equipped with Heidenhain pouches and the acid secretions from these pouches over a 5-hour period were compared before and after the administration of alloxan. There was a definite increase in acid secretion from the pouches in the alloxan-treated animals in the fasting state as compared to prealloxan studies. This increase was more marked and more apparent when they were allowed water and still more marked following the stimulus of a standard meat meal.

Alloxan, in the dosage given, results in a significant rise in acid secretion from Heidenhain pouches.

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DISCUSSION

DR. EDWIN H. ELLISON (Columbus, Ohio).—In retrospect, our alloxan diabetic animals used previously in preparing experimental chymostilosis disease all developed acute gastroduodenal ulcerations. However, we did not treat these as scientific observations.

Dr. Mayo's findings tend to discount the idea that diabetes protects against ulcer and, at the same time, they support a recent finding at our own institution in a survey of 20,000 consecutive autopsies where we found incidences of gastroduodenal ulceration in 500 diabetics (approximating 7.4 per cent). They also support our clinical impression and those of others that peptic ulceration occurring in diabetic patients is usually very intractable.

This also gives me an opportunity to give you a progress report on what has turned out to be the Zollinger-Ellison tumor registry. Total cases number 72. The concensus is that the tumor is a delta cell. One-third are multiple. One-third are malignant. Two-thirds are in the body and tail of the pancreas. Unless the tail or body is resected, 10 or 15 per cent of them cannot be found. Four patients have recently come to our attention with apparent control of their ulcer diathesis with removal of the tumor alone. The majority of new cases, unfortunately, have been removed not by the clinical surgeon but by the autopsy surgeon.

THE CARDIAC CONDUCTION SYSTEM IN CONGENITAL ANOMALIES OF THE HEART

Studies on Its Embryology, Anatomy, and Function

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DISTURBANCES of the cardiac conduction system are frequent and serious complications of intracardiac surgery. Efforts to prevent such occurrences have been hampered by the lack of exact knowledge on the location of the conduction system in relation to certain cardiac defects. The purposes of this study were (1) to locate the atrioventricular conduction system in the human embryo, particularly during the period of development of the ventricular septum, the endocardial cushions, and the conus ridges, (2) to establish the position of the conduction tissue in relation to various intracardiac defects, and (3) to evaluate the factors related to the occurrence of heart block during and after intracardiac surgery.

MATERIALS AND METHODS

Gross dissection of fresh and formalin-fixed hearts was performed by the method described by Widran and Lev.[35] The atrioventricular node and bundle were consistently identified in 8 normal adult hearts. However, in 11 hearts with high ventricular septal defects, the atrioventricular node and bundle could be identified with certainty in only 3 by the gross dissection technique.

The most consistently satisfactory method of identifying the conduction system in anomalous hearts involved reconstructions from serial sections of the area surrounding intracardiac defects. Following the photographing and sketching of specimens, a block of tissue was removed which included the ostium of the coronary sinus, the inferior portions of the atrial walls and atrial septum, the superior portions of the ventricular walls and ventricular septum, and the attached fibrous ring of the heart. This block of tissue completely encircled the defects in the region of the membranous portion of the ventricular septum and the atrioventricular septum. The tissue was sectioned in a direction corresponding approximately to the anteroposterior plane of the body. The sections were stained with either hematoxylin and eosin or Masson's triple stain. Following histologic study, graphic reconstructions were made from projected sketches of the slides.

Seven human hearts were studied by this method. Included in the group were 4 cases of ventricular septal defects and one each of the following: persistent atrioventricular canal, persistent ostium primum, and complete transposition with intact septum.

Eight human embryos, varying in crown-rump length from 11 to 41 mm., were studied. The embryos were serially sectioned in a transverse plane, and by this method a coronal section of the heart was obtained because of the transverse position of the embryonic heart.

Supported in part by Grant H-3545, National Institutes of Health, U. S. Public Health Service.

Read at the Annual Meeting of the Society of University Surgeons, Boston, Mass., Feb. 13-15, 1958.

OBSERVATIONS

Serial sections of 2 human embryos, 16 and 22 mm. in crown-rump length, were technically adequate for histologic study. In the 16 mm. specimen (Fig. 1) the dorsal endocardial cushion had not fused with the formative ventricular septum. At the top of the ventricular septum was a compact group of cells, more closely packed and more darkly stained than the surrounding areas. This appeared similar to the specimens described and illustrated by Walls[33] and identified as the atrioventricular bundle, although Walls mentions that compactness and position, rather than specific histologic properties, were the bases of his identification. Sections posterior to Fig. 2 showed the bundle beneath the endocardial cushion, in continuity with atrial musculature above and ventricular musculature of the septum below.

Sections of the 22 mm. human embryo (Fig. 2) showed a round, dense structure, identified as the atrioventricular bundle, in the superior portion of the ventricular septum. In this embryo the endocardial cushions had fused with the ventricular septum.

Two hearts were studied which showed defects related to a failure of fusion of the dorsal endocardial cushions with the ventricular septum. One of these was a membranous ventricular septal defect (Figs. 3-5); the second was a persistent atrioventricular canal (Fig. 6). In both of these specimens the atrioventricular bundle was identified in the subendocardial position along the posteroinferior margin of the ventricular defect. A similar location of the bundle was noted in 3 cases of ventricular septal defect dissected grossly.

Two hearts with single ventricles were studied. Serial sections were made of the entire posterior wall of the heart, and in neither could a true atrioventricular bundle be identified, although areas of apparent muscular continuity between atria and ventricles could be identified. Electrocardiographic studies had shown no abnormality of conduction in either case.

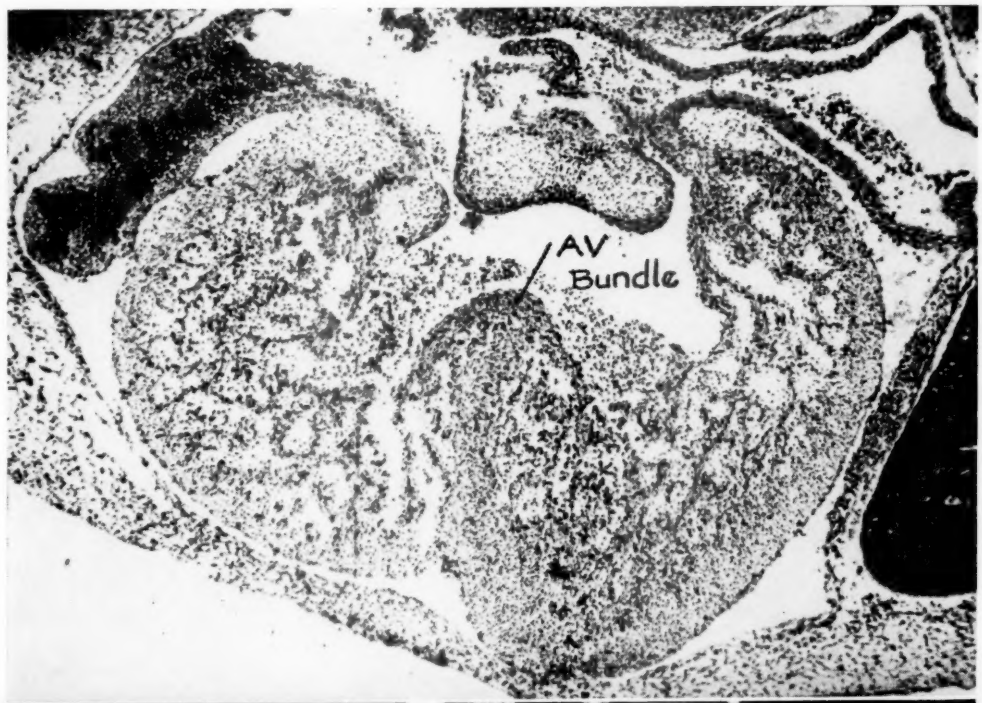
Two specimens were studied which showed anomalies apparently unrelated to the embryologic fusion of the dorsal endocardial cushion with the ventricular septum, although the malformations were found in this area. One showed complete transposition with an intact ventricular septum and the other specimen showed a defect high in the muscular portion of the septum (Fig. 7). In both cases the atrioventricular node and bundle lay in the normal positions.

One specimen of the ostium primum type of atrial septal defect was studied (Fig. 8). The atrioventricular node and bundle lay in the normal anatomic location. However, only a thin rim of rudimentary atrial musculature separated these structures from the atrial cavity.

DISCUSSION

In 1845 Purkinje[22] observed in sheep hearts a peculiar type of muscle fiber which now bears his name. His,[15] in 1893, published a description of the atrioventricular bundle, and since that time a considerable body of information has accumulated on this specialized tissue.

Fig. 1.



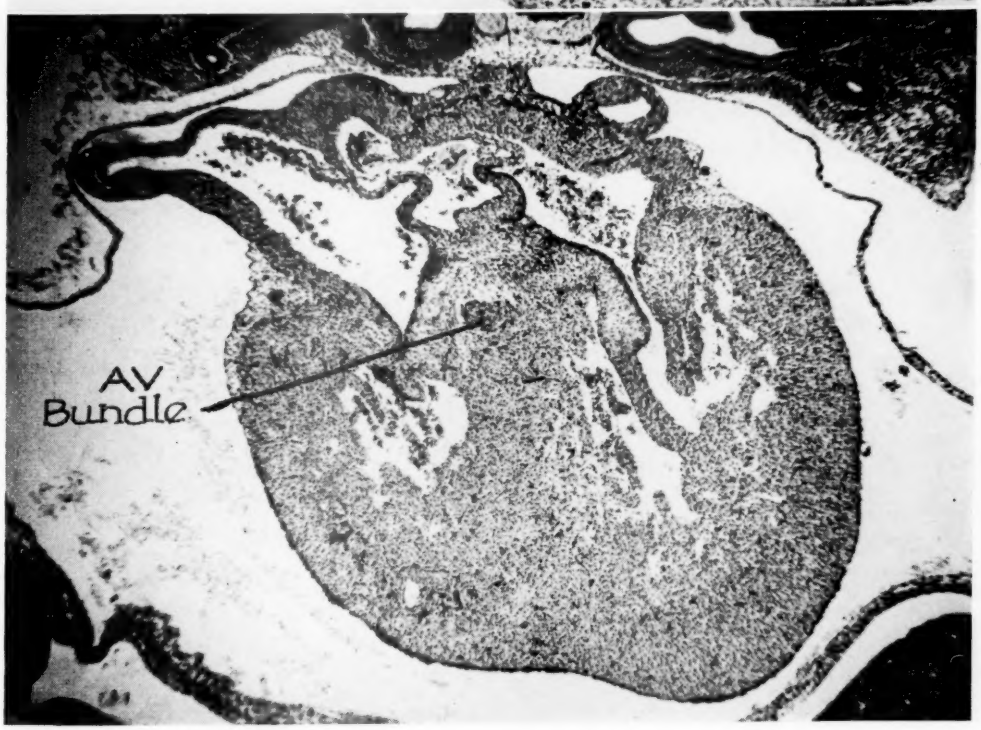


Fig. 2.

Fig. 1.—Photomicrograph of the heart of a 16 mm. human embryo, showing a compact group of darkly stained cells, identified as conduction tissue, at the top of the developing ventricular septum.

Fig. 2.—Photomicrograph of the heart of a 22 mm. human embryo, showing the atrioventricular bundle as a round, discrete structure in the ventricular septum.

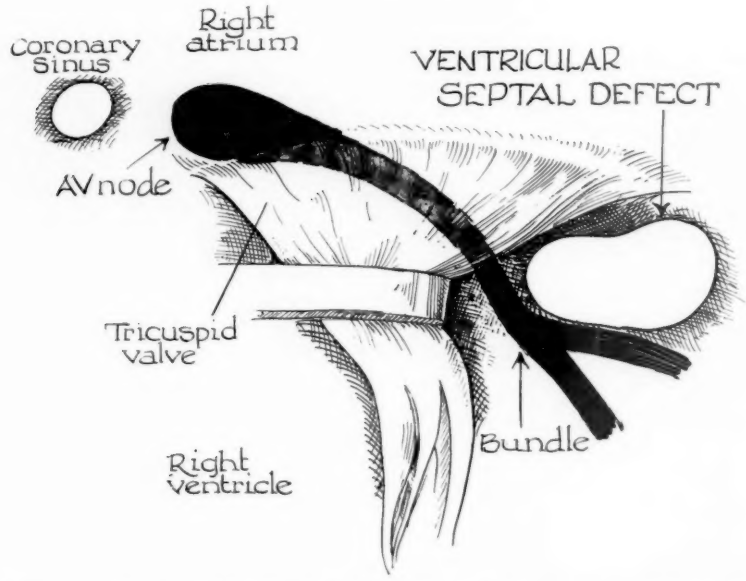


Fig. 3.—Diagram showing the location and course of the atrioventricular node and bundle in a case of membranous ventricular septal defect. The bundle lies subendocardially along the posteroinferior aspect of the defect.

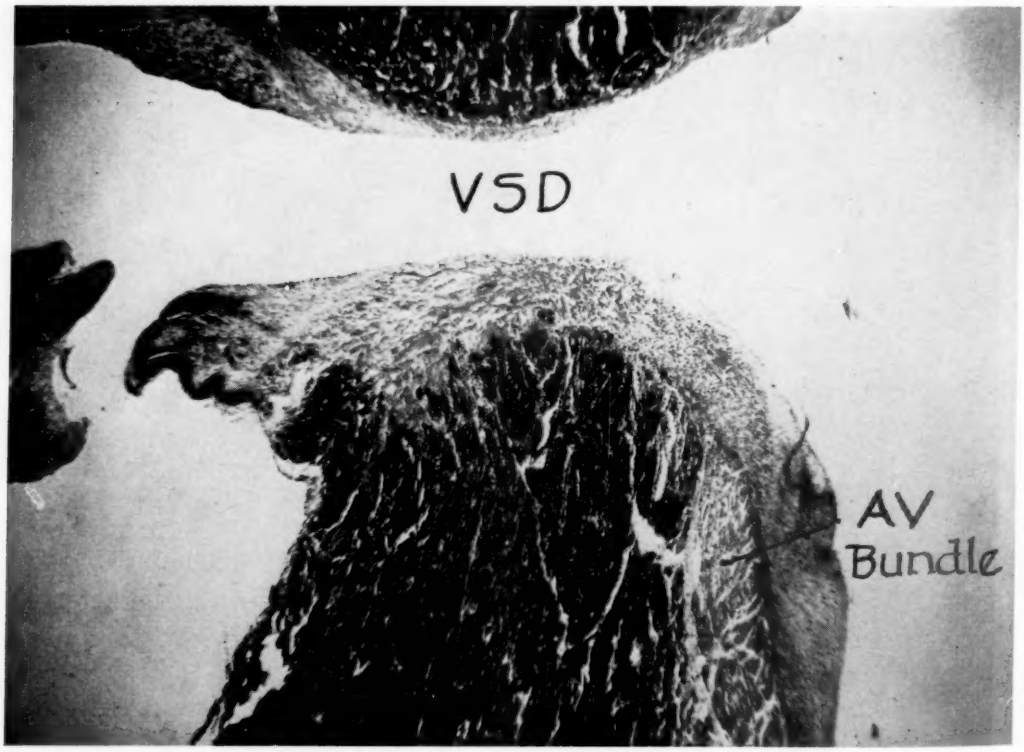


Fig. 4.—Photomicrograph of a sagittal section of the specimen shown in Fig. 3. The bundle lies on the right side of the septum, beneath the endocardium.

The development of the conduction system has been investigated extensively,[10, 24, 27, 33] and the atrioventricular bundle has been identified in 7 and 8 mm. human embryos.[21] However, except for isolated studies in cases of

Fig. 5.—Higher power photomicrograph of the section shown in Fig. 4. The conduction bundle is the pale staining tissue between the thickened endocardium and ventricular muscle.

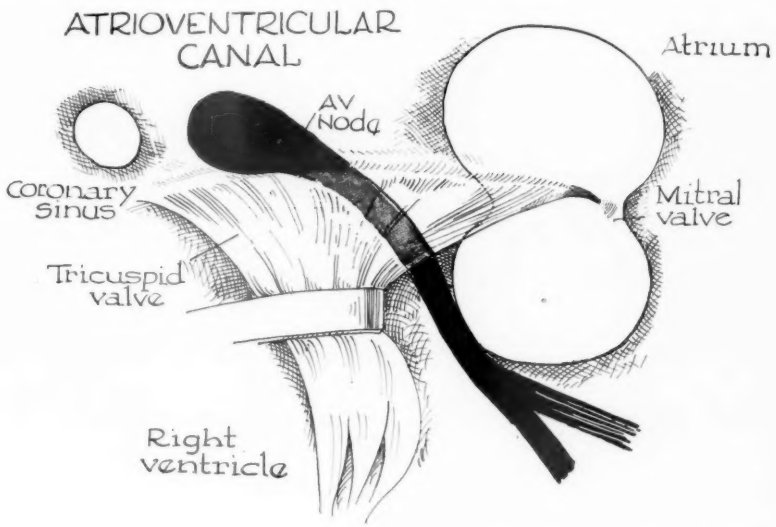


Fig. 6.—Diagram showing the location of the conduction system in a case of persistent atrioventricular canal.

congenital heart block,[34, 36-39] there has been scant information[16] on the anatomy of the conduction system in congenital anomalies of the heart until recent reports have appeared.[23, 30]

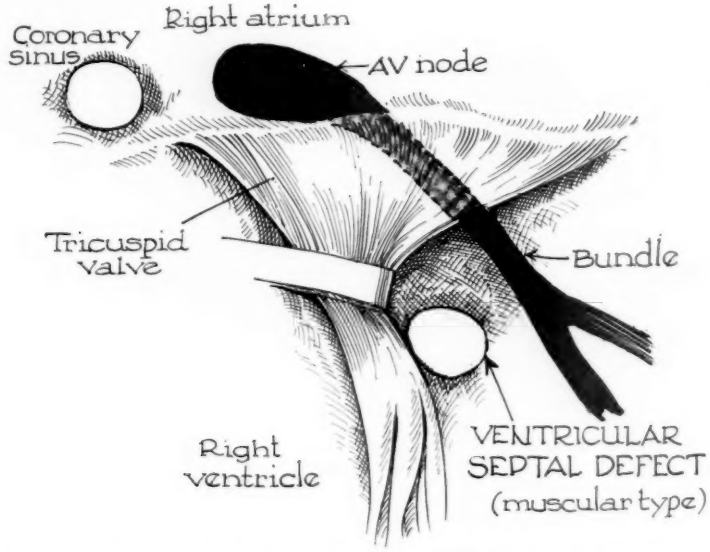


Fig. 7.—Diagram showing the conduction system in a heart with a defect high in the muscular ventricular septum.

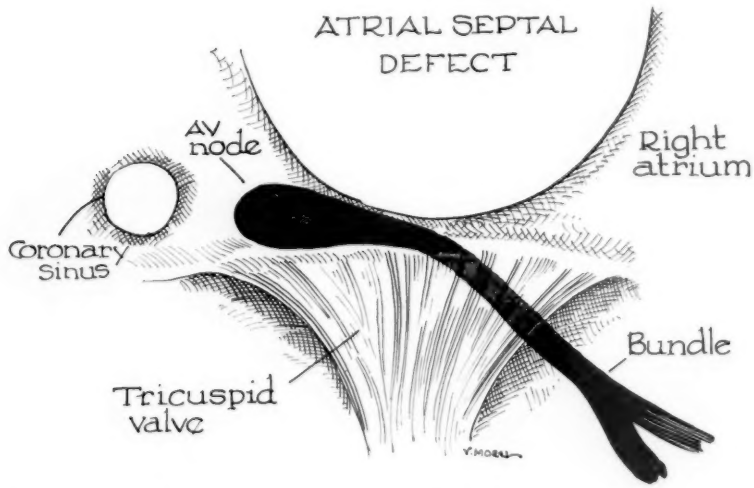


Fig. 8.—Diagram showing the location of the conduction system in a case of persistent ostium primum. Only a thin rim of muscle separates the atrioventricular node and bundle from the atrial cavity.

The recent development of intracardiac surgery has aroused renewed interest in the anatomy of congenital heart disease, and clinical experience has demonstrated the importance of conduction disturbances as a complication of intracardiac surgery. In an early series[8] of 20 cases of ventricular septal defect repaired by intracardiac operations, 9 instances of conduction disturbances were recorded. Such complications appear to be less frequent in recent series,[17, 20] but injuries to the conduction system remain one of the major hazards of intracardiac surgery. Cooley, Kirklin, and Harshbarger[2] state that

"knowledge of the exact anatomic location of the bundle of His in the presence of persistent common atrioventricular canal has long been needed, for an inadvertently placed stitch through or around the bundle may lead to complete heart block with fatal termination."

A complex series of embryologic events occurs in the area which the atrioventricular bundle normally occupies. The endocardial cushions fuse with the ventricular septum, and the truncus arteriosus is divided by the spiral septum and the conus ridges. The atrioventricular bundle arises in the posterior wall of the atrial canal and reaches the apex of the developing ventricular septum (Fig. 9). The bundle may be identified before the ventricular septum has

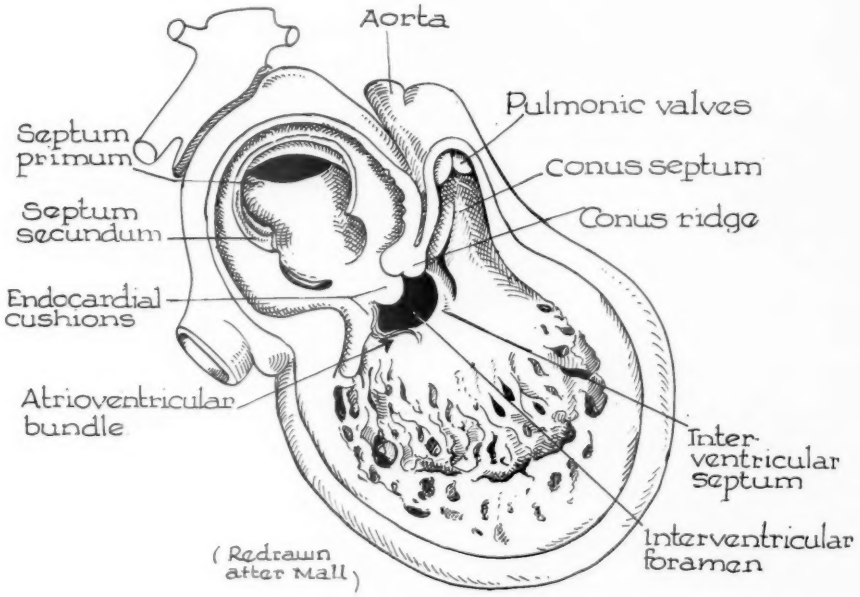


Fig. 9.—Drawing of the developing human heart, showing the position of the conduction bundle along the posteroinferior margin of the defect between the developing ventricular septum and the dorsal endocardial cushion.

fused with the endocardial cushions, and in such embryos the position of the bundle is similar to that seen in congenitally deformed hearts with membranous ventricular septal defects and persistent atrioventricular canals. In these anomalous hearts, the bundle lies along the posteroinferior aspect of the ventricular septal defect. In cases of complete transposition with intact septum, the embryologic defect is believed to be related to abnormal development of the spiral septum of the truncus arteriosus. The ventricular septum and endocardial cushions develop normally, and the position of the atrioventricular bundle is normal. Similarly in cases of atrial septal defects and muscular ventricular septal defects, the course of the atrioventricular bundle appears normal.

The anatomy of the conduction system in cases of single ventricle has not been clearly defined. In one case previously reported[36] and in 2 cases of this study, no true atrioventricular bundle could be identified, although occasional areas of muscular continuity were found.

Although the existence of specialized conduction tissue in man has been questioned,[11-14] there is now considerable evidence that such a structure exists.[3, 18, 29] Furthermore, recent physiologic studies by Davies[5] have confirmed that isolated injury to the specialized tissue results in delayed conduction.

The etiology of heart block in intracardiac surgery is not entirely clear. Certainly in many cases the disturbance in conduction appears to be related to direct injury of the atrioventricular bundle. However, in some cases heart block has occurred in the absence of manipulations in the area of the conduction tissue or even after the operation has been completed. Furthermore, the rates of recovery from operative heart block are extremely varied. Such instances suggest that factors other than direct injury are involved, such as posttraumatic edema or interference with the blood supply to the atrioventricular node and bundle. Lillehei[20] has observed 11 cases of complete heart block among patients having ventricular septal defects closed, and all occurred in patients with severe pulmonary hypertension. He suggests that the lowered "myocardial reserve" in such patients may predispose to heart block. Effler[9] has suggested that myocardial anoxia may be a causative factor in heart block occurring during intracardiac surgery. It has been demonstrated that ligation of the vessels supplying the ventricular septum in dogs frequently results in disturbances in conduction,[6] although the role of local ischemia of the conduction tissue in heart block has not been fully evaluated.

Methods to prevent injury to the conduction system at operation ideally involve identification of the atrioventricular bundle. In the beating heart, such injury is apparent immediately by the appearance of disordered cardiac beat grossly and heart block by electrocardiography. Normal rhythm may be restored in most cases by removal of the suture. However, in the arrested heart, no such system of monitoring is available. Methods of locating the conduction tissue in situ by the use of an exploring electrode or by staining have not proved satisfactory.

From the surgical standpoint, several anatomic factors deserve emphasis. In most cases of defects in the region of the membranous ventricular septum, the atrioventricular bundle lies along the posteroinferior rim of the ventricular defect. The conduction tissue lies immediately beneath the endocardium, and in the cases studied sutures could not have been placed superficial to the bundle in this area. Another anatomic feature of importance is that the bundle approaches the defect from the right, and, after the bundle divides, the right branch persists as a single bundle branch whereas on the left an arborization of the conduction tissue occurs. For these reasons, sutures placed on the left side of the septum, as Lam[19] has suggested, are less likely to involve the conduction bundle.

SUMMARY

1. Heart block remains one of the more serious and frequent complications of intracardiac surgery.

2. In membranous ventricular septal defects and related anomalies, the conduction bundle lies subendocardially along the posteroinferior aspect of the defect, a position which it occupies in embryonic life.

3. Because the bundle approaches the defect from the right and because the left bundle branch is a less discrete structure than the right, repair of the defect utilizing the left side of the apex of the septum is less likely to involve the conduction system.

4. There is evidence that factors other than direct trauma to the bundle, such as ischemia or edema, may be responsible for certain cases of heart block.

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THE TREATMENT OF CONGENITAL AORTIC STENOSIS WITH VALVOTOMY DURING CARDIOPULMONARY BYPASS

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THIS paper presents our experiences with the operative treatment with cardiopulmonary bypass of 12 consecutive patients having congenital aortic stenosis. Keener focus on this lesion has recently been made necessary with the development of means of operating under direct vision with hypothermia or extracorporeal circulation. Earlier treatment with dilators introduced through the ventricle without direct vision was not generally satisfactory because of the fibrous type of stenosis encountered and because of the danger of producing aortic insufficiency.[5, 10] Our interest in surgical treatment of this lesion has recently heightened because of the sudden death of children with few preceding symptoms or signs other than electrocardiographic abnormalities and because of the increasing conviction that fibrous stenosis in children progresses to calcific stenosis in adults with worsened prognosis and decreased possibilities of adequate surgical treatment.

CLINICAL FINDINGS

There were 11 males and 1 female in the series, which is in excess even of the usual high male-female ratio recorded in the literature[1, 4, 5, 11] (Table I). The ages at operation ranged from 3 to 19 years; one patient was under 5 years, one between 5 and 10 years, 6 between 10 and 15 years, and 4 over 15 years. The patients had been followed preoperatively in the Harriet Lane Home Cardiac Clinic for between 1 and 19 years; 6 patients had been followed for over 5 years. It was therefore possible to watch the development of electrocardiographic changes over the years and to correlate them with the onset of symptoms.

Cardiac Murmur.—A cardiac murmur was detected during the first year of life in all but 3 patients in whom it was heard at 2, 3, and 5 years, respectively. By the time the patient came to operation, the murmur and thrill were maximal in the aortic area in all cases and the murmur could be recognized to be of the stenotic type, reaching a crescendo before the second sound rather than being of uniform intensity throughout systole as in ventricular septal defect. In at least 2 patients followed for several years, the localization of the

Aid by Grant 1326 from The National Heart Institute.

Read at the Annual Meeting of the Society of University Surgeons, Boston, Mass., Feb. 13-15, 1958.

*John and Mary Markle Scholar in Medical Science.

TABLE I. CLINICAL FINDINGS IN 12 PATIENTS WITH CONGENITAL AORTIC STENOSIS

NUMBER	INITIAL	AGE	SEX	SYMPTOMS	PHYSICAL FINDINGS		X-RAY (CARDIO-THORACIC RATIO)	ECG	SITE OF STENOSIS*
					MURMUR	THRILL			
1	D.P.	13	M	Palpitation, pain, dizziness, dyspnea	Grade III systole	Strong	53	LVH	V
2	R.T.	18	M	Exertional dyspnea	Grade III systole	Strong	50	LVH; ST ↓	V
3	T.D.	3	M	Dyspnea, recurrent bronchitis	Grade III systole	Moderate	57	Incomplete R.B.B.B.	V
4	K.S.	10	F	Fatigue, exertional dyspnea	Grade III systole	Moderate	52	LVH; ST ↓	V
5	E.H.	10	M	Fatigue	Grade IV systole	Strong	49	LVH; ST ↓	V
6	P.Q.	8	M	Palpitations	Grade III systole	Strong; diffuse	56	LVH; ST ↓; bigeminy	SV
7	A.F.	14	M	Exertional dyspnea	Grade IV systole	Strong	50	LVH; ST ↓	V
8	W.P.	19	M	Exertional dyspnea, syncope	Grade III systole	Moderate; diffuse	50	LVH; ST ↓	SV
9	R.G.	17	M	Dyspnea, dizziness, pain	Grade III systole	Moderate	45	LVH	V
10	R.M.	19	M	None	Grade IV systole	——	—	LVH; ST ↓	V
11	M.G.	14	M	Exertional dyspnea	Grade IV systole Grade I diastole	Strong	55	LVH; ST ↓	SV
12	G.B.	11	M	Pain, syncope, dyspnea	Grade IV systole	Moderate	50	LVH	SV

*V = valvular, SV = subvalvular.

murmur to the aortic area did not become apparent until over 3 years of age, and erroneous diagnoses of ventricular septal defect or mitral insufficiency were temporarily entertained. In 2 patients the thrill, although well felt in the aortic area, was unusually diffuse, and in both of these the stenosis was subvalvular in type. The aortic second sound was diminished or absent in all cases and was not a useful sign of the site of the stenosis. A Grade I early diastolic murmur indicative of mild aortic incompetence was present in 2 patients, both with subvalvular stenosis.

Dyspnea.—Most patients were completely asymptomatic during infancy and early childhood, although slight exertional dyspnea was often commented on in retrospect by the parents. A characteristic clinical story was given by D.P. (Case 1, Table I), who was followed from the age of 7 years. A cardiac murmur was discovered at birth, but he had no symptoms except for mild exertional dyspnea until 12 years. He then had a fairly sharp increase in exertional dyspnea and occasional dizziness on strenuous exercise. He also had mild substernal pain described as tight or squeezing in character coming on after playing games or other exertion. Following the onset of these symptoms, his activities were restricted and games forbidden, although he continued to go to school. The symptoms disappeared following these restrictions, but his electrocardiogram, which had shown increasing left ventricular hypertrophy since the age of 7 years, continued to deteriorate and by 13 years showed ST depression and flattening of T waves over the left precordial leads. Operation was undertaken at 14 years, and 9 months postoperatively the electrocardiogram had returned to normal and the cardiothoracic ratio had decreased from 53 to 45 per cent.

Fatigue and Dyspnea.—This progression, the onset of fatigue and dyspnea 2 or more years before the appearance of severe electrocardiographic changes, was fairly typical. Exertional dizziness or syncope was present in 4 patients and in 3 of them was associated with mild substernal pain on exertion. Four patients complained only of dyspnea on strenuous exertion, and one child (Case 5) complained only of fatigue. He had a systolic gradient across the aortic valve of 120 mm. Hg, illustrating the paucity of symptoms that may accompany a severe degree of aortic stenosis. One child (Case 3), the youngest in the series, had an atypical history and electrocardiogram. Following an attack of pneumonia and congestive failure at 5 weeks of age, he remained persistently dyspneic even on mild exertion with frequent recurrent bronchitis, persistent cardiomegaly, and clinical and catheter evidence of pulmonary hypertension (pulmonary artery pressure, 50/17 mm. Hg). Although the murmur and thrill were typical for aortic stenosis, the presence of incomplete right bundle branch block on the electrocardiogram and an apical mid-diastolic murmur together with left atrial enlargement led to anxiety that there was an accompanying mitral valve lesion. No evidence of this was found at left heart catheterization or at operation and his signs, symptoms, and heart size have all regressed satisfactorily in the 4 months following aortic valvotomy.

Palpitations.—Palpitations due to numerous ventricular extrasystoles and later to bigeminy were the presenting symptom in one child of 8 years who had the highest pressure gradient in the series. The bigeminy disappeared after aortic valvotomy and was thought to have originated from impending left ventricular failure.

Roentgenography and Fluoroscopy.—Roentgenography and fluoroscopy showed mild-to-moderate left ventricular enlargement in all cases. Great cardiomegaly was not present; the cardiothoracic ratio ranged from 47 to 57 per cent. Poststenotic dilatation of the aorta immediately above the valve was recognized in only 3 of the 8 patients with valvular stenosis, and its absence therefore did not necessarily indicate a subvalvular stenosis.

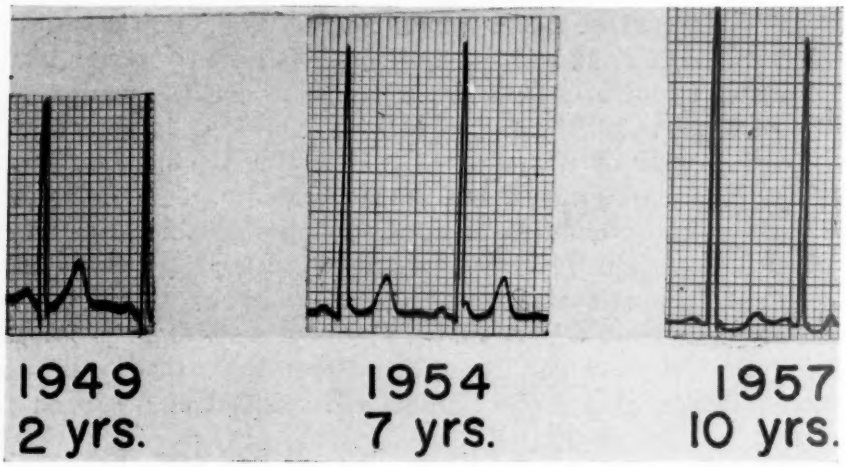

Fig. 1.—The progressive electrocardiographic changes in Patient K. S., over a 9-year period with typical progressive left ventricular hypertrophy in Lead V_6.

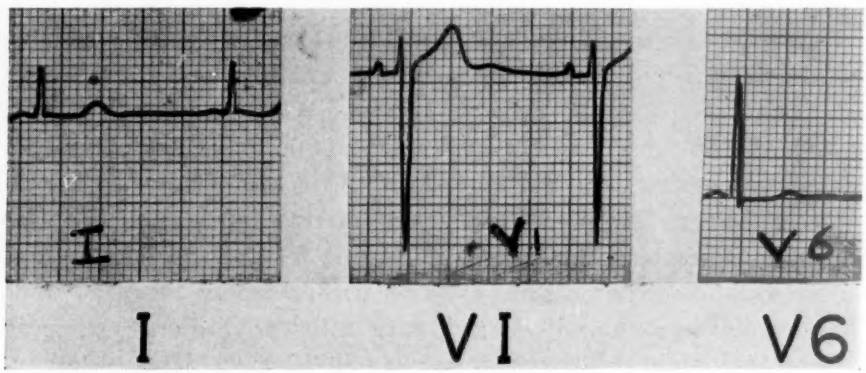

Fig. 2.—The electrocardiographic changes with a small pressure gradient (40 to 60 mm. Hg.) across the aortic valve in Patient R. G., aged 17 years.

Electrocardiography.—The electrocardiogram showed left ventricular hypertrophy in all patients except Case 3 previously discussed. The left ventricular hypertrophy was progressive over the years (Fig. 1), and all the patients by the time operation was undertaken showed varying degrees of ST depression and flattening or inversion of the T waves over the left precordial

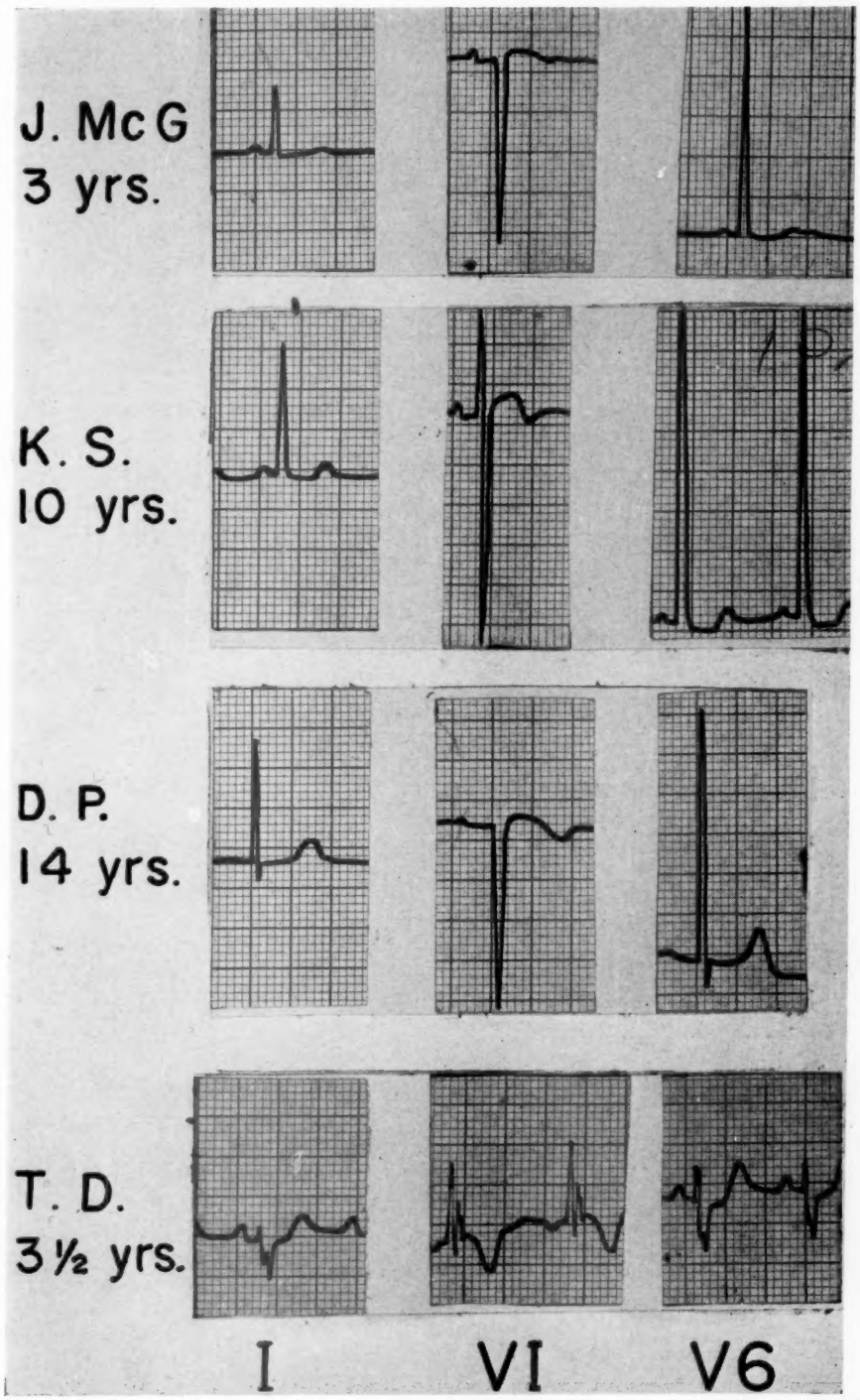

Fig. 3.—The electrocardiographic changes with a moderate pressure gradient (60 to 100 mm. Hg.) across the aortic valve are shown.

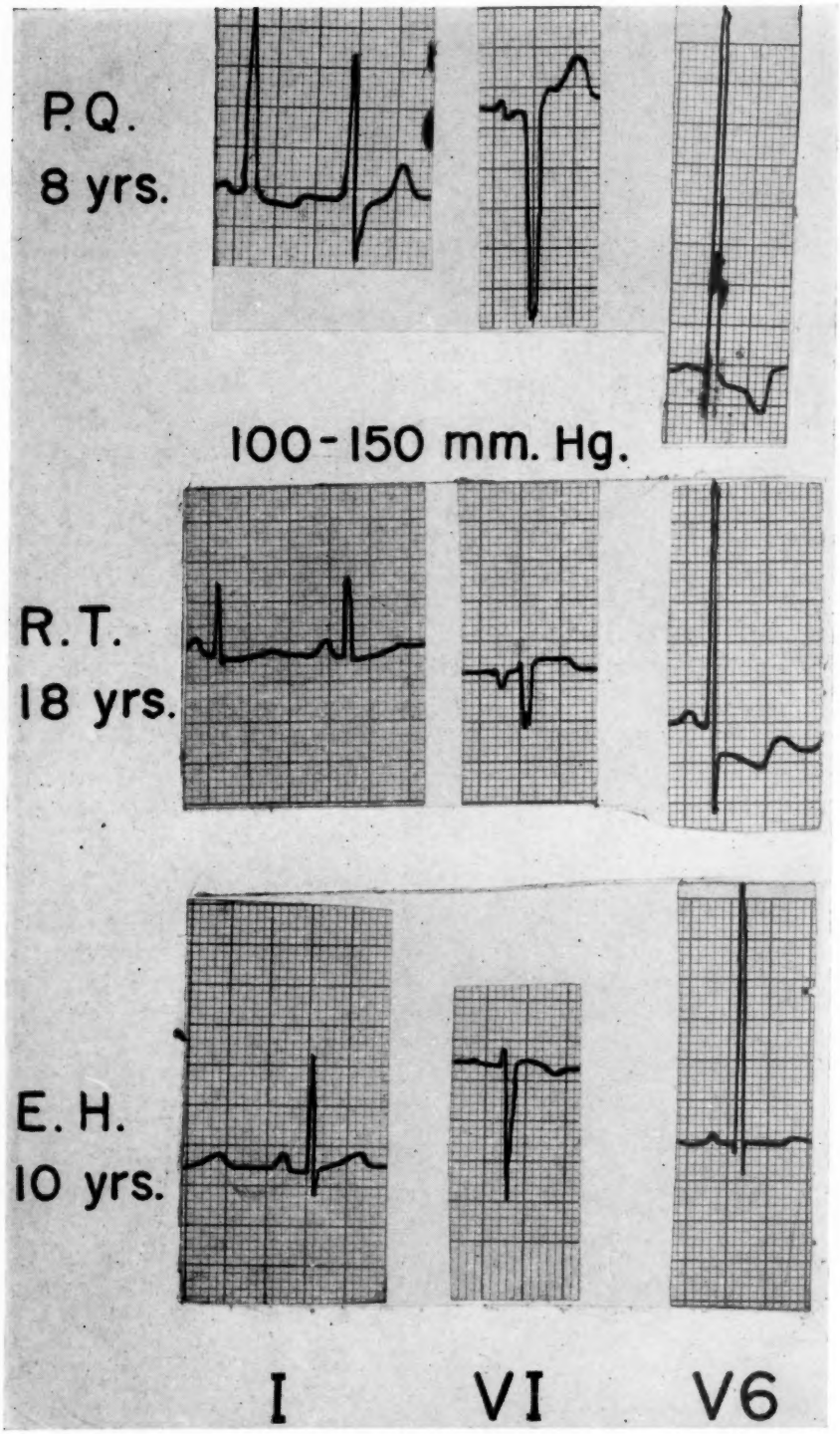

Fig. 4.—The electrocardiographic changes with a large pressure gradient (100 or more mm. Hg.) across the aortic valve are shown.

leads. The degree of ST depression and T-wave changes was least in the one patient with the systolic gradient across the valve of only 50 mm. (Fig. 2), was moderate in the majority of patients, all with a gradient of 60 to 100 mm. (Fig. 3), and was most marked in the 4 patients with systolic gradients exceeding 100 mm. Hg (Fig. 4). The electrical axis was normal and the electrical position was vertical or semivertical in 9 of the 12. In only 2 patients, both with subvalvular stenosis, was the left ventricular hypertrophy associated with left axis deviation and a horizontal heart.

Heart Catheterization.—Right heart catheterization was performed in only 2 patients in the present series. Left heart catheterization was undertaken in 5 patients (Cases 1, 3, 9, 11, and 12) and was successful in 4. The procedure was felt indicated if there was a suspicion of additional mitral stenosis, as in Case 3, if there was associated aortic insufficiency (Case 11), or if there was a discrepancy between the symptoms and the electrocardiographic changes (Case 9). The gradients obtained at catheterization correlated well with those at surgery. The procedure was not felt necessary as a routine, because the clinical diagnosis was often clear and the electrocardiogram was a good guide to the severity of the stenosis.

Angiocardiography.—Angiocardiography in an attempt to localize the site of the stenosis was performed in 3 patients. In all, the procedure correctly indicated a valvular stenosis. Recently we have ceased using this technique since the subaortic stenosis encountered has been a diaphragm below the valve readily treated by the present method of surgery.

Errors in Diagnosis.—All 12 patients were diagnosed preoperatively as having aortic stenosis but in only 1 of the 4 subvalvular cases was the exact site of stenosis recognized. In retrospect the diffuse quality of the systolic thrill, the presence of left axis deviation, and the presence of a greater degree of cardiomegaly than usual may all be possible pointers to a subvalvular site, but the series is too small to be dogmatic. Over the past 8 months during which the series of operations has been undertaken, one patient has been subjected to thoracotomy for aortic stenosis in whom no aortic disease could be found at operation. She survived the thoracotomy, and although the diagnosis remains obscure the electrocardiogram has returned to normal. Another patient was sent to surgery with a diagnosis of combined aortic and pulmonary stenosis. Both of these conditions were present and in addition he had a ventricular septal defect which had not been diagnosed either clinically or at catheterization. This patient died at operation and exhibited a long area of infundibular narrowing of the left ventricle, which seems to be rare as an isolated malformation in the heart.

METHODS

No special preoperative care was given. Pentothal, succinylcholine, and nitrous oxide were used as anesthetic agents. Relative hyperventilation was used throughout the operation to produce a slight alkalosis. The chest was usually incised in the right third intercostal space with transverse division of the sternum; the left pleural cavity was not entered. Median sternotomy in the last 4 patients provided equally good exposure. The ascending

aorta was moderately dilated in most patients, and a strong thrill was palpable near the aortic valve. The aorta in the patients with subaortic stenosis was similar to those with valvular stenosis. The left ventricular pressure was measured with a strain gauge by a catheter inserted through the left atrium into the ventricle; simultaneously, arterial pressure was measured through another catheter in the iliac artery.

The screen oxygenator developed by Gibbon[7] and modified by Gaertner and Kay[6] was used in all instances.* After the patient was heparinized (3 mg. of heparin per kilogram of body weight), a single steel cannula (one-half inch in inside diameter, with a multiholed, rounded end) was placed in the right atrium through the appendage for the removal of venous blood, and a steel cannula of appropriate size was inserted into the femoral artery for arterial return. After cardiopulmonary bypass was begun, the ascending aorta was clamped and cardiac arrest was produced by the rapid injection of 2 or 4 per cent solution of potassium citrate in oxygenated blood. A 5 cm. incision that extended to the area of the right coronary artery was made in the ascending aorta. Good exposure of the aortic valve was obtained. Blood that continued to flow from the left ventricle during the procedure was removed by gentle aspiration and returned to the pump-oxygenator.

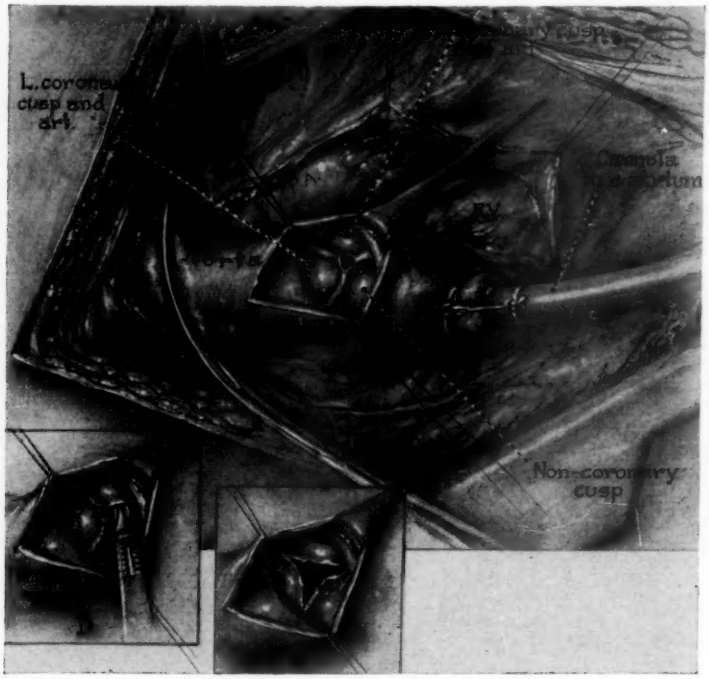

Fig. 5.—A valvular aortic stenosis has been exposed with an incision in the ascending aorta. The aorta is clamped distally. Venous blood is being carried through the cannula in the right atrium to the pump-oxygenator. *B*, A fused commissure is incised with a knife. *C*, Two commissures have been completely opened; the third commissure has not been completely opened in order to avoid insufficiency.

The stenosed valves were surprisingly similar in all 8 patients (Fig. 5). The diameter of the stenosed orifice varied from 2 to 6 mm. Fused valve commissures could be easily identified, although the valve cusps were often of unequal size. The commissure between the right and the left cusps was usually completely fused, that between the right and noncoronary cusps was partly fused, and the commissure between the left and noncoronary cusps often was not fused at all. Calcification was found in only one patient, our oldest, a

*Made by the Mark Company, Randolph, Mass.

19-year-old boy who had known of a murmur for many years.* The fusion of the cusps in this patient resembled the pattern seen in other children, but the thickened deposits in the valve resembled the usual adult calcified aortic stenosis.

The commissures were opened with a small-bladed knife (Bard-Parker, No. 15). Particular care was taken to incise in the center of the fused commissure so that each cusp would have a thickened edge to provide a broader area for coapting and thus to lessen the hazard of aortic insufficiency. Each commissure was usually opened to the aortic wall. After the development of moderate aortic insufficiency in one patient, the commissure between the right and the left aortic cusps was only partly opened in 2 subsequent ones with mild stenosis. In spite of this, however, one of these developed moderate regurgitation. The valve orifice was enlarged to a diameter of 15 to 20 mm. in each patient; after valvotomy, the outflow tract of the left ventricle was digitally examined for a subaortic stenosis. The method of incision of the fused commissures and the extent to which they should be opened remained an individual problem for each patient. One of the main advantages of cardiopulmonary bypass was that time was available for careful observations and decision.

Subaortic stenosis was found in 4 patients whose aortic valves were normal (Fig. 6). The stenotic opening was 3 to 4 mm. in diameter and 1 to 3 cm. proximal to the valve. Rongeurs were used to enlarge the opening to a diameter of about 2 cm.

Fig. 6.—The aortic valve has been exposed as in Fig. 5. A subvalvular stenosis has been found 1 cm. proximal to the aortic valve. The normal aortic valve cusps have been retracted to expose the subvalvular stenosis.

Particular care was taken to avoid injury to the base of the aortic leaflet of the mitral valve, the ventricular septum, and the aortic valve cusps. A large pressure gradient remained in one patient in whom only the stenosed fibrous tissue was excised. In subsequent patients some of the left ventricular muscle often was removed; this step should probably be done routinely because of the muscular hypertrophy associated with the stenosis. As Brock[2] has stressed in regard to right ventricular infundibular stenosis, there often is a fibrous band at the point of stenosis but adjacent muscle is also usually thickened.

Air was displaced from the aorta by blood draining through the left ventricle while the incision was being sutured with 4-0 continuous silk; the aorta was unclamped when

*Treated during a visit to the Royal Prince Alfred Hospital, Sydney, Australia.

closure was complete. Cardiac contractions began a few seconds later in 6 patients and were soon followed by a normal cardiac rhythm. The cardiac rhythm in 6 patients began with a strong, coarse ventricular fibrillation. This was easily converted to a sinus rhythm with 1 to 4 electric shocks, 110 to 220 volts and a current duration of 0.1 second being used. No subsequent cardiac arrhythmia developed. Heparin was neutralized with protamine (2 to 3 mg. per kilogram of body weight). After removal of the cannulae, the incisions in the right atrium and femoral artery were sutured. Any leaks or gaps in the aortic closure were reinforced with interrupted sutures. Delayed bleeding from the aortic closure in 2 patients has made us particularly careful of this point.

Postoperative care did not differ significantly from that following other cardiac operations. Penicillin, streptomycin, and erythromycin were routinely given for several days. Five patients with signs of myocardial insufficiency were digitalized following operation.

RESULTS

All of the patients survived operation and were discharged in good condition. The postoperative follow-up ranges from 1 to 6 months. Postoperative bleeding required a second thoracotomy a few hours after operation in 4 of the 12 patients (Cases 4, 6, 8, and 10, Table I). The bleeding came from the internal mammary vein in one patient, the aortic suture line in 2, and, much to our surprise, the puncture site in the right atrial wall in one patient, where a 20-gauge needle had been inserted to measure intracardiac pressures. No defect of blood coagulation was found in any of the patients. No further bleeding occurred after the second thoracotomy.

One patient (Case 6, Table I) with subaortic stenosis developed positive blood cultures for *Pseudomonas* on the fourth postoperative day during an episode of 105° F. fever and severe diarrhea. The same organism was isolated from the stool. Antibiotics were given for 12 days. All subsequent blood cultures were sterile. He was afebrile after the twelfth postoperative day and was discharged from the hospital 16 days later. Two days after discharge he developed petechiae and splenomegaly and, although blood cultures were sterile, was treated for probable bacterial endocarditis with 4 weeks of intensive antibiotic therapy with a good result. Three other patients had an unexplained febrile course for as long as 25 days after operation; numerous blood cultures were sterile.

The data obtained at operations are tabulated in Tables II and III. The duration of cardiopulmonary bypass ranged from 12 to 30 minutes, with a flow rate between 1.5 and 3.8 L. per square meter of body surface per minute (Table II). The valve orifices were 2 to 6 mm. in diameter before valvotomy and 16 to 20 mm.* afterward. The systolic pressure gradient across the aortic valve ranged from 42 to 122 mm. Hg in the 8 patients with valvular stenosis. The pressure gradient was over 213 mm. Hg in one patient with subaortic stenosis (Table III). The pressure gradient after valvotomy in 6 patients ranged from 11 to 60 mm. Hg. Less gratifying changes occurred in 2 of the patients with subaortic stenosis. A gradient of 146 mm. remained in one, and the gradient increased 27 mm. in another patient after resection (R.P., Table III). These

*Estimated from digital palpation after valvotomy.

TABLE II. DATA FROM 12 OPERATIONS FOR AORTIC STENOSIS

NUMBER AND INITIAL	DURATION OF CARDIOPULMONARY BYPASS (MIN.)	DURATION OF CARDIAC ASYSTOLE (MIN.)	FLOW RATES* (ML./MIN./SQ. M. BODY SURFACE)	ESTIMATED SIZE OF STENOTIC ORIFICE (MM.)		VENTRICULAR FIBRILLATION	AORTIC INSUFFICIENCY AFTER VALVULOTOMY	BLOOD PRESSURE (MM. HG)	
				BEFORE VALVULOTOMY	AFTER VALVULOTOMY†			PREOP.	POSTOP.‡
1. D. P.	15	10	2,500	3	20	No	No	105/80	110/80
2. R. T.	18	10	2,800	4	20	No	No	98/84	100/70
3. T. D.	20	11	2,800-3,800	2-3	16	No	No	90/70	100/70
4. K. S.	19	14	3,200	2-3	20	No	No	95/70	110/75
5. E. H.	20	12	2,500-3,100	3	16	Yes	Moderate	110/80	110/40
6. P. Q.	30	24	2,800	3-5	19-20	Yes	§	95/70	110/80
7. A. F.	15	10	1,800	4-5	16	Yes	No	100/70	120/70
8. W. P.	19	17	2,300	4	20	Yes	§	110/85	120/70
9. R. G.	18	10	—	5-6	20	Yes	Moderate	110/70	110/20
10. R. M.	19	—	—	—	20	Yes	No	—	—
11. M. G.	14	10	1,500	4-5	20	‖	§	90/70	120/64
12. G. B.	23	16	18-3,500	3-4	20	No	§	92/78	102/80

*Estimated from pump rate per minute.
†Estimated from diameter of finger inserted through valve orifice after valvulotomy.
‡Blood pressure at last postoperative examination.
§Subaortic stenosis.
‖Cardiac arrest produced with Mecholyl.

gradients remained despite enlargement of the stenotic orifices to about 20 mm. in diameter in each patient. In the last patient a more generous resection of the adjacent muscle was done with gratifying disappearance of the gradient.

TABLE III. AORTIC-LEFT VENTRICULAR PRESSURE GRADIENTS BEFORE AND AFTER OPERATION (MM. HG)

NUMBER AND INITIAL	BEFORE OPERATION			AFTER OPERATION		
	LEFT VENTRICLE	AORTA	SYSTOLIC GRADIENT	LEFT VENTRICLE	AORTA	SYSTOLIC GRADIENT
1. D. P.	179/16	109/59	70*	—	—	—
2. R. T.	200/5	85/69	115	130/9	84/56	46
3. T. D.	>180/11	124/74	>56	96/0	102/81	0
4. K. S.	199/26	133/95	66	99/11	88/69	11
5. E. H.	254/11	132/85	122	112/6	84/40	28
6. P. Q.	>320/—	107/80	>213	260/10	114/76	146
7. A. F.	140/58	98/35	42†	—	—	—
8. W. P.	130/13	96/62	34	156/0	95/53	61
9. R. G.	135/4	83/64	52	105/6	87/63	18
10. R. M.	—	—	140	—	—	60
11. M. G.	218/24	102/59	116	157/20	105/69	52
12. G. B.	173/18	110/65	63	91/12	94/42	0

*Preoperative left heart catheterization.
†Validity of pressures uncertain.

Ventricular fibrillation developed during recovery from potassium-induced cardiac arrest in 6 patients. In each instance it was easily converted to a sinus rhythm with 1 to 4 electric shocks.

Aortic insufficiency of a moderate degree developed in 2 patients after valvotomy. The patient with probable bacterial endocarditis has a residual murmur of mitral incompetence, not recognized preoperatively. This may be an associated congenital lesion. Most patients have shown a decrease in intensity of the systolic murmur and thrill and a return of pulse pressure to normal levels.

DISCUSSION

Indications for Operation.—These patients were operated upon because symptoms of fatigue, dypnea, dizziness, or chest pain were associated with progressive electrocardiographic changes of left ventricular hypertrophy or strain. A good correlation was found between these electrocardiographic changes and the pressure gradient across the stenosed orifice found at operation. It is interesting that one patient had no symptoms and was able to go spear fishing and play 4 hours of tennis. He was operated upon for severe electrocardiographic changes alone and at surgery had a systolic gradient across the valve of 140 mm. Hg with considerable calcification. Only slight cardiac enlargement was present. Symptoms were often not severe. This policy of early operation is well supported by the findings of Braverman and Gibson,[1] Marquis and Logan,[10] and Downing,[5] all of whom found few symptoms preceding sudden death in their patients, except for moderate dyspnea, dizziness, or chest pain, and electrocardiographic changes of left ventricular hypertrophy or strain. The incidence of sudden death in their patients was 8, 15, and 8 per cent, respectively.

Although it has not been a consideration in these patients, the presence in Case 10 of a thickened calcified valve, thought to be superimposed on a congenital stenosis but resembling the calcified adult lesion, has reinforced our conviction that the lesion in adults often represents progression from a congenital defect. Prevention of the surgically unsuitable adult lesion by early operation in childhood seems a logical step if the safety and efficacy of treatment in childhood can be established.

On the other hand, because of the benign prognosis in some cases of congenital aortic stenosis, we do not at present think surgery is justified in an asymptomatic patient with minimal or mild left ventricular hypertrophy and no ST or T-wave changes because of the possibility of causing aortic insufficiency and the absence to date of long-term postoperative follow-up.

Method of Operation.—Congenital aortic stenosis has been treated by dilatation,[3, 5, 10] by direct valvotomy under hypothermia,[3, 9, 11, 12] and by direct valvotomy with extracorporeal circulation. The fibrous type of stenosis found in these patients would not seem suited to simple dilatation as the fibrous tissue may stretch instead of actually separating, or, if the commissure is tightly fused, a cusp may be torn with resulting severe insufficiency. Open valvotomy under hypothermia is limited by the short period of time available; circulatory occlusion longer than 5 minutes is hazardous because of the irritability of the hypertrophied left ventricle. We have been impressed that several minutes may be necessary to be certain of the exact position of the fused commissures, especially when the valve cusps are of unequal size. In addition, a careful incision of the fused commissure has seemed necessary in order to separate the commissure along its center and thus minimize insufficiency. Auscultatory evidence of insufficiency was present after valvotomy in 2 of the 8 patients treated. However, 4 of 8 patients treated by Swan[12] and 3 of 4 patients treated by Morrow[11] had some insufficiency after valvotomy performed under hypothermia.

An adequate length of time is equally necessary for excision of a subaortic stenosis. Injury to the aortic leaflet of the mitral valve, the ventricular septum, or the aortic cusp may occur. These hazards can be lessened by careful visualization in a relatively empty heart.[3]

Pressure Gradients.—A satisfactory reduction in pressure gradient was obtained in all patients with valvular stenosis. The high residual gradient in one patient with severe subaortic stenosis (P.Q., Table III) may have been due to hypertrophy of the left ventricular muscle, partial excision of which might have resulted in further reduction of the gradient. Another patient with subaortic stenosis (W.P., Table III) had a small pressure gradient that was little influenced by enlarging the stenotic opening from 4 to about 20 mm. Yet the stenosis in this patient had caused an acute episode of left ventricular failure a few years before operation before physical activity was markedly restricted. These inconsistencies in pressure gradient across the stenosis clearly indicate the need for a simultaneous determination of blood flow in evaluating the amount of stenosis present. Such determinations have recently been made by Morrow and associates.[11]

The pressure gradient was completely abolished in the last patient treated for subaortic stenosis (Case 12, Table I). This was accomplished by a more radical excision of left ventricular muscle at the site of the stenosis than had been done in previous patients.

Ventricular Fibrillation.—Ventricular fibrillation during recovery from potassium-induced cardiac asystole occurred in 50 per cent of the patients. The fibrillation was easily corrected with electrical defibrillation. Defibrillation might be a more difficult problem if severe aortic insufficiency were present because regurgitation of blood from the aorta would overdistend the left ventricle and possibly retard its recovery. Investigation is needed of other cardioplegic agents which might lessen the incidence of fibrillation in this disease. Mecholyl was used in 2 patients, but cardiac contractions resumed after 1 to 2 minutes of arrest. We prefer complete asystole in the heart deprived of its blood supply. Aortic valvotomy without cardiac arrest would seem hazardous because of the rapid development of severe myocardial ischemia. Gott and Lillehei found a 90 per cent mortality in 15 dogs subjected to 15 minutes of aortic occlusion during cardiopulmonary bypass.[8] We have not used retrograde perfusion of the coronary sinus clinically, as potassium-induced arrest has seemed to us more satisfactory.

SUMMARY

Twelve patients with congenital aortic stenosis were successfully treated by incision of the stenotic area during cardiopulmonary bypass with potassium-induced cardiac arrest. The stenosis was valvular in 8 patients and subaortic in 4. Ventricular fibrillation in 6 of the 12 patients was easily treated with electrical defibrillation. Fused commissures were present in each stenosed valve; these were opened by incision along the center of the fused commissure. The type of commissural fusion was surprisingly similar in all 8 patients with valvular stenosis. Moderate insufficiency followed valvotomy in 2 patients. The results indicate that aortic valvotomy may be done with cardiopulmonary bypass with low risk and satisfactory results. Early operation should be considered in children with symptoms and progressive changes in the electrocardiogram of left ventricular hypertrophy with ST depression and flattening or inversion of T waves over the left precordium.

The advice and encouragement of Dr. Alfred Blalock and Dr. Helen Taussig were of much help in the treatment of these patients.

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Discussion

DR. DENTON A. COOLEY (Houston, Tex.).—The treatment of aortic stenosis represents a significant problem to those of us who are engaged in open heart surgery. For example, in the 310 cases that we have operated upon using the pump-oxygenator, we have had 26 cases of aortic stenosis.

(Slide.) In this slide, you can see that in our congenital stenoses of which there were 13—incidentally, we also had 13 acquired cases—in the congenital group, 5 were valvular, 7 were subvalvular, and one was combined valvular and subaortic stenosis.

You will note that we had no deaths in the congenital group, whereas in the calcific acquired stenoses our results were not as good. We had 5 deaths among 13 cases. You are all well aware of the complicated problems which you run into with the acquired group. One of the patients in this group was 65 years old. That did not seem to be a handicap because he fell into the recovered group.

(Slide.) In our 13 cases, we had the unique experience of having 3 patients who were less than 6 months of age. Congenital aortic stenosis in the very young patient presents an unusual problem in that these patients have congestive failure and are completely pulseless in many instances. In fact, all 3 of our patients were completely pulseless, and it made a very dramatic case in which the pulses were completely restored throughout.

The average age in our patients was 8 years. There were a number of associated defects which we considered to be of secondary importance to the aortic stenosis. One patient had a ventricular atrial defect, the left ventricular to the right atrial communication, which was corrected at the same time. Another had an infundibular pulmonic stenosis which we had not suspected prior to cardiac catheterization. We resected the subpulmonic stenosis and then, using a transaortic approach, resected the subaortic stenosis.

One interesting patient had aortic sclerosis. This is something which I have not looked into in the literature and I do not know how frequently it occurs. In this patient, in opening the ascending aorta, we found that the effective lumen was about 4 mm. in diameter. This happened to be a patient about 2 years of age and, in that patient, we carried out a thromboendarterectomy of the ascending aorta from the sinus of Valsalva well around into the transverse aortic arch and involving the innominate and carotid arteries. This patient also had a valvular pulmonic stenosis which was opened and made a satisfactory recovery.

Another patient had cardiac tamponade. This patient, 36 hours previously, had a left ventricular puncture as a diagnostic procedure and actually had cardiac arrest on the ward 12 hours before the scheduled operation. We opened his chest on the ward in unsterile conditions, evacuated the pericardial tamponade, and then set up the pump-oxygenator and operated upon the patient that night. He made an uneventful recovery after the aortic valvulotomy.

(Slide.) The question arises in Dr. Spencer's cases about the efficacy of using potassium arrest. We have gone through several stages in our own work on the use of cardioplegic agents and you can see here that we had 60 cases, our initial group, without aortic occlusion. We had a ventricular fibrillation rate of 8 per cent. Here with the potassium arrest, we had an incidence of fibrillation of 24 per cent and recently, since we have ceased using potassium in any of our cases, our ventricular fibrillation incidence is 16 per cent.

Therefore, we have more or less discarded the use of potassium because of this high incidence of ventricular fibrillation and certainly Dr. Spencer might consider that since he had a high incidence himself.

(Slide.) In some of the larger patients with strictly left-sided lesions of the heart, we have been cannulating them in this manner here, using a single venous cannula up through the inferior vena cava and up into the superior vena cava. That has seemed to minimize the amount of dissection necessary in setting up the bypass.

DR. ROBERT S. SHAW (Boston, Mass.).—Our experience at the Massachusetts General Hospital with aortic stenosis is embarrassingly small and is confined to middle-aged patients with posttraumatic calcific aortic stenosis.

However, we have made one observation that I think is important. The principal difficulty that we have had with these patients is in the recovery phase, after the valvulotomy has been completed. The left ventricle seems to recover more slowly than the right side and there is pulmonary edema. We found, working with dogs first and, finally, with one patient, that we could overcome this difficulty which I think is more prominent in patients who have coronary disease, too, by putting an accessory catheter in the left auricle, which will decompress the pulmonary side and give a left heart bypass during the recovery phase. It has been possible with this to have a period of very good coronary perfusion, to avoid the pulmonary hypertension that gives pulmonary edema, and, also, to avoid the left ventricular distention which I think makes ventricular recovery much more difficult. It has worked very well in dogs and was very successful indeed on one case.

DR. SPENCER (Closing).—As I mentioned in the presentation, we have preferred to use potassium because of the findings of Dr. Lillehei and his workers that were very convincing to us that, if the aorta were occluded for 15 minutes, there was a 90 per cent mortality in dogs. I suspect that, with Dr. Cooley's usual speed, his occlusion time is 3 to 4 minutes in most of his patients.

CHANGES IN BODY COMPARTMENTS FOLLOWING OPERATION

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(From the Laboratories for Surgical Research, Department of Surgery, The New York Hospital–Cornell Medical Center)

THE loss of body fat stores has been recognized for some time as a significant fraction of the loss of body weight after operation. The measurement of changes in body fat have not been satisfactory because determinations of body fat in surgical patients have required indirect methods. A recent report from these laboratories[5] demonstrated that a modification of Newburgh's formula for the change in body fat may be applied with advantage in the analysis of weight changes in postoperative patients. The present report is an extension of these studies. Fat changes determined by this modified formula are compared with change in body fat determined by other methods.

METHODS

The patients in this study consist of 5 women who presented themselves with complaints arising from gastric lesions, 2 benign and 3 malignant. In Patient No. 1 a leiomyoma of the gastric fundus required wedge resection. In Patient No. 2 a partial gastrectomy was performed for a gastric ulcer. Patient No. 3 had carcinoma of the gastric antrum for which a radical subtotal gastrectomy was performed. In the remaining 2 patients (Nos. 4 and 5) gastric carcinoma was treated by total gastrectomy and adjacent resection. Gastrointestinal continuity was restored by interposing a jejunal segment between the esophagus and duodenum. Abstracts of the clinical course of these patients are appended.

The patients were admitted to the metabolic section of the surgical pavilion service. Balance studies were carried out for nitrogen, potassium, and sodium. Intake error was minimized by the use of a homogenized oral formula which was analyzed by batch for nitrogen, potassium, sodium, water, fat, and carbohydrate. Postoperative intravenous alimentation consisted of appropriate isotonic solutions of glucose and electrolytes except in the case of Patient No. 2 whose postoperative intake of calories and nitrogen was maintained intravenously with a fat emulsion (IV-Lipomul*) and a protein hydrolysate (Aminosol). Patients Nos. 1 and 2 received the anabolic hormone Nilevar. The weight of all ingesta and excreta were carefully noted each day. The patients were accurately weighed at the same time each day by the same observer. The principles which governed the balance studies and analytic methods used in this laboratory have been outlined previously in detail.[1]

Dilution studies were done at intervals on each patient prior to operation and during the recovery period. Total body water was determined from deuterium oxide dilution.

This study was supported by grants from The Astor Foundation, The New York City Cancer Committee, and The Lillia Babbit Hyde Fund.

Read at the Annual Meeting of the Society of University Surgeons, Boston, Mass., Feb. 13-15, 1958.

*Supplied through the courtesy of The Upjohn Company, Kalamazoo, Mich.

Extracellular fluid was measured by the dilution of radiosulfate ($S^{35}O_4$). The use of this latter isotope for the study of extracellular fluid has been discussed by Walser[15] and Ryan.[11] These studies were performed early in the morning under basal conditions.

At zero time a preinjection blood sample was obtained and then approximately 50 Gm. of 99.8 per cent D_2O and 50 microcuries of $S^{35}O_4$* prepared for parenteral use were injected intravenously from tared syringes. Blood samples were then obtained at 1, 2, 3, and 4 hours after injection. Deuterium oxide determinations were done from the 2- and 3-hour samples. The measurement of this isotope in the plasma samples followed that of Schloerb and Moore.[12] After the first deuterium oxide dilution study was done, residual D_2O in the preinjection samples was determined for each subsequent total body water determination.

Plasma radiosulfate content was determined by the method described by Swan.[14] The $S^{35}O_4$ was isolated as the barium sulfate precipitate from the trichloracetic acid filtrates obtained from the 1, 2, 3, and 4-hour blood samples. Where indicated this was also carried out on the preinjection blood sample. The radiosulfate solution which was used for injection was suitably diluted and precipitated in the same fashion. The resulting values for plasma sulfate levels were plotted on semilogarithmic paper. The straight line curve for these points was then extrapolated back to zero time and the resultant value, the theoretical plasma sulfate at zero time, was used in calculating the dilution of the known amount of $S^{35}O_4$ injected. The resultant value was not corrected for plasma water or the Donnan equilibrium. Any residual isotope present in the preinjection sample was taken into account in these calculations.

Chemical methods of analyses of formula and excreta were similar to those described in a previous study.[1]

CALCULATIONS

The method for determination of metabolized fat was suggested by Newburgh[8] whose calculations have been modified in view of more recent data. His original formula for determining the components of insensible weight loss, solving for fat (F), may be expressed as: (1) $F = \frac{IL - (2.18C + 12.26N)}{3.93}$, where IL is the insensible weight loss, C is the carbohydrate content of the diet which is assumed to be completely burned, and N is the nitrogen output by the patient taken as an expression of the protein burned.

The derivation of the formula depends upon the observation of Soderstrom and DuBois[13] and Benedict[3] that the insensible water loss (IW) bears a constant relationship to the calories burned: (2) $\frac{IW \times 0.58}{calories} = 25\%$. This formula indicates that the calories which are utilized in the vaporization of water from the skin and lungs comprise 25 per cent of the total calories burned. The data on which this formula was based were collected from calorimeter studies.

A more recent calorimeter study by DuBois[4] shows that the proportion of calories expended in the heat of vaporization is not fixed but increases with the environmental temperature and that at 30° C. the vaporization of water for both men and women employs 31 per cent of the calories. The subjective comfort temperature for DuBois's subjects varied between 28° and 31° C. We measured the underclothing temperature in 40 bed patients on basal

*The $S^{35}O_4$ was obtained from Oak Ridge National Laboratories on authorization of the Atomic Energy Commission.

conditions by placing a screened bulb thermometer between skin and pajamas. The mean underclothing temperature in this group was found to be 32.2° C. (range 30.2° to 34.2° C.).

Since the temperature of the immediate environment of the skin of our bed patients on the surgical service was similar to the comfort zone temperature of DuBois's subjects and in light of his data that at this temperature range 31 per cent of the calories expended are used for the vaporization of water, we have revised equation (2) to read: (3) $\frac{IW \times 0.58}{\text{calories}} = 31$ per cent.

Insensible water loss may be expressed as: (4) $IW = IL - (CO_2 - O_2)$ where IL is insensible weight loss and may be determined from the total intake, total output, and change of weight. The factor $(CO_2 - O_2)$ is determined from the metabolic mixture and depends upon the O_2 utilized and the CO_2 produced for each gram of carbohydrate, protein, and fat metabolized. When IW in formula (4) is substituted in formula (3) and the caloric equivalent of carbohydrate (from the intake) and protein (from the nitrogen output) metabolized is placed in the denominator, and the formula is solved for fat (now the only unknown), the revision of formula (1) results in: (5) $F = IL - \frac{(2.6C + 15.1N)}{4.9}$. Thus, the facts needed for the calculations of fat are weight of total intake, weight of total output, body weight change, carbohydrate burned, and nitrogen excreted.

To determine the body fat burned (BF-Newburgh), fat in the intake is subtracted from the total fat burned. A negative value signifies a storage of fat. In the present study, the BF was determined for each day of study. Because daily total intake and output, especially during intravenous feedings, vary considerably the calculated insensible loss (IL) was occasionally found to be abnormally high or low. As these extraordinary values for IL were believed to be artifacts, the IL for 2 or 3 days were averaged. This accounts for the rather smooth curves describing fat changes in Figs. 1-5.

The figure for the daily lean tissue change was determined by multiplying the nitrogen balance by 30.

Water changes, not accounted for by the water of lean tissue or fat changes, were determined by 2 different methods based upon the dilution techniques. The $S^{35}O_4$ space is believed to give a fairly close approximation of the extracellular space.[14, 15] If water changes are extracellular, they should be reflected in this space. The $S^{35}O_4$ space was corrected for the extracellular fluid water in lean tissue, taken as 20 per cent of the change in lean tissue, and for that in fat taken as 10 per cent of the change in fat tissue. The D_2O space or the total body water change was corrected by subtracting from it 80 per cent of the lean tissue change and 10 per cent of the fat tissue change, the approximate water content of these 2 components of weight change. The corrected space will hereafter be referred to as $S^{35}O_4$ sp. corr. and D_2O sp. corr., respectively. They were plotted cumulatively on the days which the dilution studies were performed (Figs. 1-5). For the analysis by periods, the

results were prorated over the periods between the dilution studies, when the days of the periods did not coincide with the days on which the dilution studies were performed.

As Newburgh has pointed out, calculations such as these on patients are subject to errors which are minimized when the study is carried out over several days. This is especially true of patients undergoing the radical changes which occur with surgery. In the analysis of the data, therefore, we have divided the data on each patient into periods of varying length from 3 to 7 days. In each the day of operation was the first day of a period. In a few instances where the space study was not done on the last day of the period, the change of D_2O on that day was estimated on the basis of the average change in the previous days.

The present study provides several alternate methods for the determination of body fat. If it is assumed that the mineral mass changes insignificantly in experiments of as short duration as the present ones, body weight change must be equal to the sum of the changes of lean tissue mass (LTM), fat (BF), and corrected water: (6) $\Delta BW = \Delta LTM + \Delta BF + \Delta H_2O$ corr. Using the 2 different expressions for corrected water change described and solving for dehydrated fat which is 90 per cent of tissue fat, the formula reads: (7) $\Delta BF\ (S^{35}O_4) = 0.9\ [\Delta BW - (\Delta LTM + \Delta S^{35}O_4$ sp. corr.)], and (8) $\Delta BF(D_2O) = 0.9\ [\Delta BW - (\Delta LTM + \Delta D_2O$ sp. corr.)]. The latter formula (8) may be calculated by subtracting from BW in the bracket the expression (6.25 N bal. + D_2O sp.), which thus avoids correcting the D_2O space for lean tissue water. Both calculations were done (Table I) and the results were similar.

Body fat itself may be determined by the formula of Pace[10] which is based upon specific gravity determination: (9) $BF(Pace) = BW - \frac{D_2O \text{ space}}{0.732}$.

As noted by Moore,[7] the application of the Pace formula for the determination of body fat is inadequate during periods of marked shifts in water. This determination, therefore, has been restricted to the preoperative total body water values and the last total body determination which was obtained after acute changes in water had subsided. Thus the values obtained by us with this method give only an over-all value for fat loss in the period following operation.

RESULTS

Observations on the daily changes in weight, lean tissue mass and fat (BF – Newburgh), and corrected water by the 2 dilution methods ($S^{35}O_4$ sp. corr. and D_2O sp. corr.) are presented graphically in Figs. 1-5. The changes are shown cumulatively with zero indicating the beginning of the study. Because each patient was a special problem and was treated differently, each will be discussed separately.

Patient No. 1 (Fig. 1).—This patient received an oral formula supplemented by intravenous Lipomul for 4 days prior to operation. After excision of a benign gastric tumor she received no nitrogen and minimal calories until

oral feedings were resumed gradually from the fifth postoperative day. The small loss of lean tissue postoperatively was probably due to 3 factors, (1) the relatively small amount of stress, (2) her depleted state, and (3) the anabolic hormone which she received for 2 days before and 7 days after operation. The loss of body fat, which continued until the end of the study, has been observed in other patients who received Nilevar and presumably reflects an additional requirement for energy in the conservation of lean body mass. There was little change in body water which was not accounted for by the changes in lean tissue and body fat. This may be noted by the curves of $S^{35}O_4$ sp. corr. and D_2O sp. corr., both of which show changes which are probably within the experimental error.

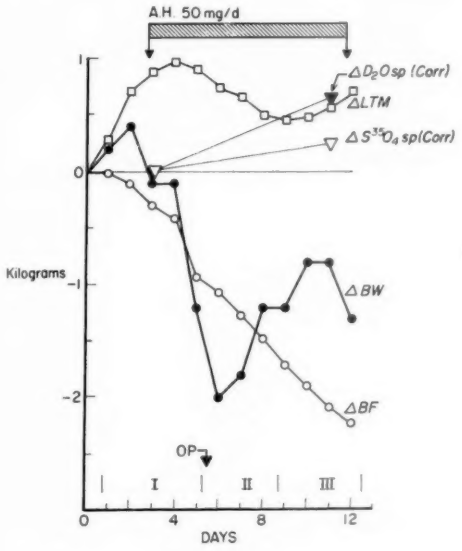

Fig. 1.

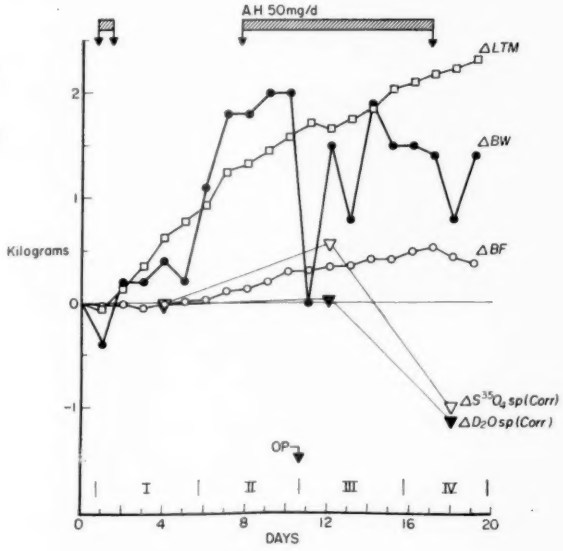

Fig. 2.

Fig. 1.—Patient No. 1. △LTM = change in lean tissue mass, △BW = change of body weight, △BF = change of body fat after Newburgh, $\triangle D_2O$ sp. (corr). and $\triangle S^{35}O_4$ sp. (corr). = deuterium space and radiosulphate space corrected for changes in lean tissue and fat tissue. Roman numerals indicate periods of study. Minimal operative trauma. AH = Nilevar.

Fig. 2.—Patient No. 2. This patient received an increased intake of calories and nitrogen in the period following operation (see appended abstract). Moderate operative trauma. See Fig. 1 for explanation of symbols.

Patient No. 2 (Fig. 2).—This patient lost 3.5 Kg. of body weight in the 5-week period prior to admission. She regained 2.4 Kg. during the 11 preoperative days on the oral formula. After operation the caloric and nitrogen intake was maintained at the preoperative level by intravenous infusions of fat emulsion and protein hydrolysate until oral feedings were resumed. She received 50 mg. Nilevar daily for 3 days before and 6 days after operation. As with Patient No. 1 there was a minimal lean tissue catabolism postoperatively, probably due to her depleted state, the hyperalimentation, and the Nilevar. The caloric intake was high and was sustained throughout the postoperative period. This resulted in a sparing of body fat. A net loss of water occurred postoperatively, as may be noted in the curves representing $S^{35}O_4$ sp. corr. and D_2O sp. corr., probably due to withdrawal of the hormone.

Patient No. 3 (Fig. 3).—The oral formula given preoperatively permitted lean tissue storage in this patient with a history of long-standing inadequate intake. In the postoperative period the intake was not supplemented and a rapid weight loss occurred. Oral feedings were resumed gradually after the third postoperative day. Fig. 3 shows that the weight loss of 2.2 Kg. in 7 days was due to the loss of lean tissue and fat in approximately equal amounts. There was a slight net gain in water according to the D_2O sp. corr., whereas the $S^{35}O_4$ sp. corr. showed a loss of water which totaled 0.8 Kg. following operation. The sum of the changes of lean tissue, fat, and D_2O sp. corr. in the postoperative period agree within 0.1 Kg. with the weight changes. However, when $S^{35}O_4$ sp. corr. is added to lean and fat tissue changes there is a discrepancy of 1.2 Kg. The significance of this finding will be discussed later.

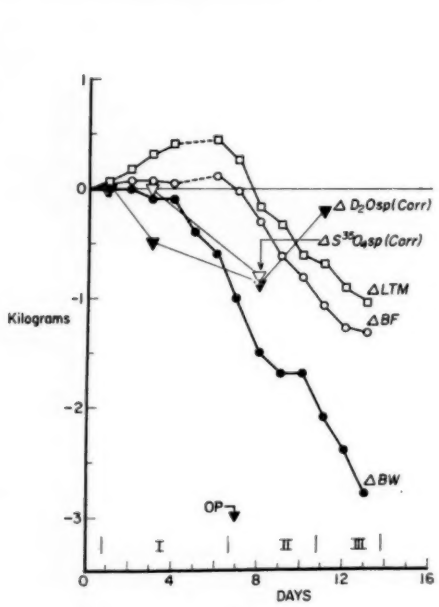

Fig. 3.

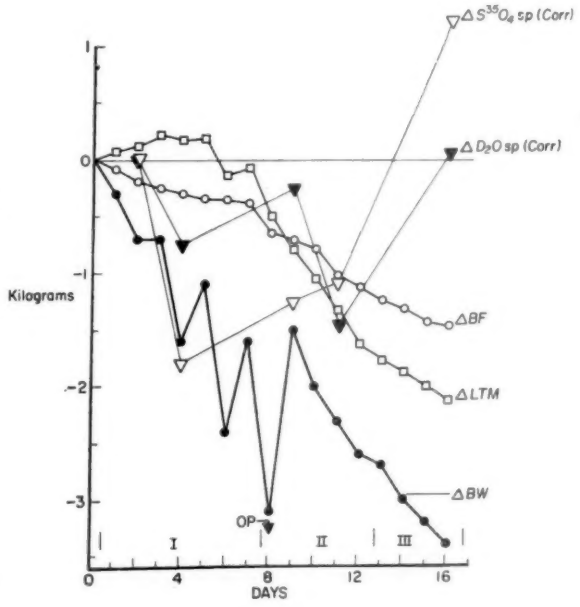

Fig. 4.

Fig. 3.—Patient No. 3. This patient administered minimal calories and nitrogen during the early convalescent period. See Fig. 1 for explanation of symbols.

Fig. 4.—Patient No. 4. Minimal caloric and nitrogen intake in the immediate postoperative period. Severe operative trauma. See Fig. 1 for explanation of symbols.

Patient No. 4 (Fig. 4).—In spite of an adequate caloric intake in the preoperative 7 days, the patient continued to lose weight, which may be accounted for in part by lean and fat tissue as seen in Fig. 4, and in part by a loss of water. The part played by the patient's advanced tumor should also be considered. Since the water loss is apparent by both the $S^{35}O_4$ sp. corr. and the D_2O sp. corr., it suggests a contraction of extracellular fluid volume.

This patient was subjected to the marked trauma of total gastrectomy. For 6 days postoperatively she received only the calories in the daily isotonic glucose solutions. Her weight loss was accounted for by the loss of lean and fat tissue. This is corroborated by the small change of $S^{35}O_4$ sp. corr. and the D_2O sp. corr. However, in the last 4 days of the study the $S^{35}O_4$ sp. corr. is

high, whereas the D_2O sp. corr. remains low suggesting that water has shifted into the extracellur space. This was corroborated by a retention of sodium noted in the balance study and the appearance of clinical edema.

Patient No. 5 (Fig. 5).—The major stress of total gastrectomy was further magnified by enterocolitis which was first recognized on the third postoperative day. The vigorous treatment for this complication included ACTH, antibiotics, and transfusions. This therapy resulted in gross water shifts which can be accounted for only partially by the components measured in the figure. The agreement in periods III and IV is especially poor. There was a loss of fat and lean tissue but a gain of weight only partly accounted for by the $S^{35}O_4$ sp. corr. or the D_2O sp. corr. This case is presented as an example of the limitations of the method in such complicated patients. One might anticipate lack of agreement when such radical changes occur so rapidly.

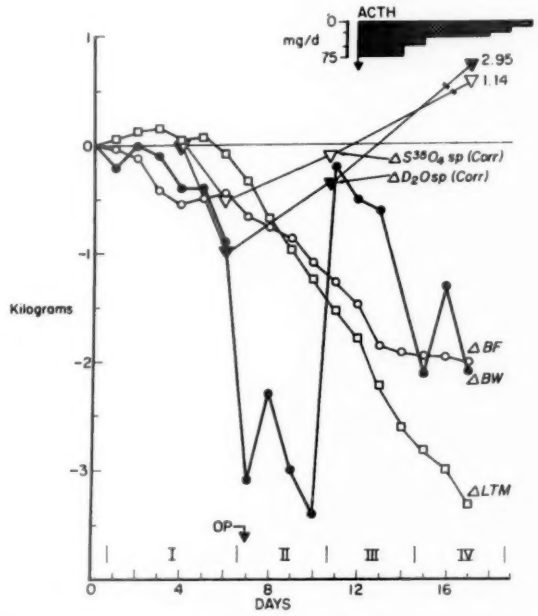

Fig. 5.—Patient No. 5. Minimal caloric and nitrogen intake in the period following operation. Severe operative trauma. Course complicated by enterocolitis. See Fig. 1 for explanation of symbols.

Table I summarizes the results of the study by metabolic periods in the 5 patients. The weight change is shown at the left. To the right are shown the calculated components of the weight changes; the lean tissue mass change, the change in $S^{35}O_4$ sp. corr. or change in D_2O sp. corr. for water, and the 3 methods for measuring body fat changes, that of Newburgh and those from the 2 methods of water, where fat is determined by difference.

There are several methods by which one may estimate the accuracy of the Newburgh calculations for body fat changes. First, one may note in the table whether the sum of the components of the weight change equals the total weight change. When the water change is minimal, as is the case in Patient No. 1, periods II, III, the lean change plus Newburgh fat change agree closely

TABLE I. COMPONENTS OF WEIGHT CHANGE

PERIOD	DAYS	ΔBW	ΔLTM (L.)	Δ $S^{35}O_4$ SP. CORR.	Δ D_2O SP. CORR.	ΔFAT BY DIFFERENCE ($S^{35}O_4$)	ΔFAT BY DIFFERENCE (D_2O)	ΔFAT (NEWBURGH)
1. I	4	-0.10	+1.00					-0.4
II	4 op	-1.10	-0.50	+0.21	+0.62	-0.7	-1.1	-1.1
III	4	-0.10	+0.20	+0.04	+0.04	-0.3	-0.3	-0.8
2. I	5	+0.20	+0.79	+0.29	-0.24	-0.8	-0.3	+0.1
II	5	+0.80	+0.80	+0.26	-0.27	-0.2	+0.2	+0.3
III	5 op	-0.50	+0.46	-0.69	-0.72	-0.2	-0.2	+0.1
IV	4	-0.10	+0.28	-1.09	-1.02	+0.6	+0.6	0.0
3. I	5	-0.10	+0.44	-0.24	-1.00	-0.2	+0.4	+0.1
II	4 op	-1.10	-1.04	-0.30	+0.90	+0.5	-0.9	-0.9
III	3	-1.10	-0.43					-0.5
4. I	7	-1.60	-0.08	-1.56	-0.82	0.0	-0.6	-0.4
II	4 op	-1.10	-0.82	+0.20	+0.22	-0.4	-0.5	-0.4
III	4	-0.80	-0.51	+1.85	-0.05	-1.9	-0.2	-0.3
5. I	6	-0.90	-0.07					-0.4
II	4 op	-2.70	-1.17	+0.25	+0.16	-1.6	-1.5	-0.6
III	4	+2.30	-1.37	+0.60	+1.57	+2.8	+1.9	-0.8
IV	4	-0.80	-0.78	+0.57	+1.24	-0.5	-1.1	-0.2

op—Operation first day of period marked op.

ΔBW—Change in body weight.

ΔLTM—Change in lean tissue mass.

Δ$S^{35}O_4$ sp. corr.—Change in radiosulfate space corrected for lean tissue and fat tissue water.

ΔD_2O sp. corr.—Change in total body water corrected for lean tissue and fat tissue water.

Last 3 columns indicate calculated fat changes for each metabolic period by method indicated at head of each column.

with the weight change. When a water change is involved, it is necessary not only to show that the Newburgh fat calculation is accurate, but also that the measurement of water is correct. Alternate methods for determination of fat are therefore required. The table shows that there is good agreement (that is, they are within 0.3 Kg. of each other) between fat (Newburgh) and fat (D_2O) in 8 of the 14 periods, whereas only 3 periods show good agreement between fat (Newburgh) and fat ($S^{35}O_4$).

Fat from the Pace formula was calculated preoperatively and near the end of the study on each patient. Data from which these calculations were made (body weight and D_2O spaces) and the resulting changes of fat are

TABLE II. DILUTION STUDIES AND FAT DETERMINATIONS (PACE AND NEWBURGH)

PATIENT	BW	$S^{35}O_4$ SPACE	D_2O SPACE	B FAT (PACE)	ΔBF (PACE)	ΔBF (NEWBURGH)
1. Preop.	67.6	14.0	31.2	25.1		
6 days postop.	66.4	14.0	31.4	23.5	-1.6	-1.6
2. Preop.	59.2	10.7	28.8	19.9		
6 days postop.	60.4	10.0	28.6	21.3	+1.4	+0.1
3. Preop.	60.0*	12.2*	33.8*	13.9*		
4 days postop.	58.3	11.0	33.0	13.1	-0.8	-0.7
4. Preop.	65.4*	13.7	32.4*	21.0*		
11 days postop.	62.0	13.4	31.0*	20.4*	-0.6	-1.1
5. Preop.	56.3*	10.2*	28.0*	18.0*		
11 days postop.	55.3	10.6	28.4	16.2	-1.8	-1.6

BW—Body weight in kilograms.

$S^{35}O_4$ space and D_2O space—radiosulfate space and total body water in liters, respectively.

B Fat (Pace)—Body fat calculated from Pace formula.

ΔBF—Change of body fat in postoperative period using Pace and Newburgh formulas.

*Average of 2 determinations.

shown in Table II. The change of fat (Newburgh) for the postoperative period is also shown for comparison. It is interesting that in 4 of the 5 patients the agreement is good when the assumptions that must be made are considered. There is no ready explanation for the poor agreement in Patient No. 2. Comparison of the 4 methods for measuring fat change in all 5 patients is represented graphically in Fig. 6.

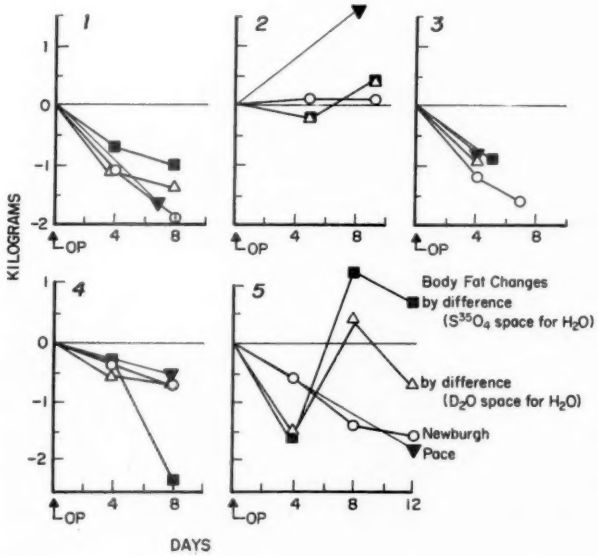

Fig. 6.—Summary and comparison of the 4 methods of determining change in body fat for the immediate postoperative period only. See text for details on calculations employed. Points in the individual curves do not coincide in each patient as the metabolic periods and the point at which the isotope studies were done did not occur on the same day.

DISCUSSION

The purpose of this study was to extend the application of a method for measurement of components of body weight change with particular reference to fat change in surgical patients. This study shows that a number of conditions influence the relative amount of fat, lean tissue, and body water which are lost following trauma. One factor is the nutritional status of the patient and the stores of lean tissue and fat that are available prior to operation. Other factors are postoperative feeding, hormone administration, and the presence or absence of complications. Patient No. 1 who received Nilevar appears to have expended approximately twice as much fat as lean tissue in the postoperative period, whereas Patient No. 3 burned about the same amount of fat as lean tissue. Patients No. 4 and No. 5 lost more lean tissue than fat.

In order to account for the weight change one must estimate changes in body water which are not due to the changes in water of lean tissue and fat. Two methods have been used for making this correction, that using the D_2O space and that using the measurement of extracellular space with $S^{35}O_4$. Each of these has been corrected for the water estimated to have been gained or lost with the gain or loss in lean tissue and fat.

The results presented here indicate that when the D_2O sp. corr. is used for water change the components of weight change are more accurately accounted for than when $S^{35}O_4$ sp. corr. is used. The importance of this finding is that when both methods are used simultaneously they become a means of studying water shifts between the cells and the extracellular fluid. Thus it is suggested that intracellular water may play a more active part in water shifts than previously thought. For example, in Patient No. 2, period I, there was a gain in body weight which was less than the sum of the gains of fat and lean tissue which indicated a loss of water. This was corroborated by a loss of D_2O sp. corr.; however, the $S^{35}O_4$ sp. corr. showed a slight gain rather than a loss and thus it is suggested that there was a loss of intracellular water. Period IV of the same patient showed small changes in weight, lean tissue, and fat, but there was a 1 Kg. loss in both D_2O sp. corr. and $S^{35}O_4$ sp. corr. suggesting an extracellular water depletion probably incident to the withdrawal of Nilevar. The weight loss of Patient No. 3, period II, accounted for only half of the value of lean and fat changes. This discrepancy was accounted for by a gain in the D_2O sp. corr. water. Since the $S^{35}O_4$ sp. corr. decreased, one may suggest that the water gain here was due to an increase in intracellular water.

These analyses are only examples of possible interpretations of the data but the conclusions cannot be definite. In view of the possible errors in balance studies, in the dilution studies, and in the assumptions required for calculating fat, it is apparent that when components of a weight change are as small as those in the present study, we are dealing with figures very close to the margin of error.

The present work must be extended to patients in whom a greater loss is expected than occurred in the present group. This would perhaps indicate more clearly the accuracy of the method. Prolonged studies by the balance method may also aid in determining the applicability of this method to the acutely ill patient. However, as noted by Moore,[7] balance studies tend to contain cumulative errors in themselves.

An understanding of the limitations of the Newburgh method of calculating fat permits better evaluation of the result. Newburgh[9] and Lavietes[6] have outlined some of the assumptions which are necessary for the calculations which may be subject to variation. They admit that the use of an average figure for vaporization may not be allowable under certain conditions and particularly in diseased patients. Furthermore any sensible perspiration or over-cooling of the skin invalidates the use of insensible loss for estimating heat production.

Many factors may influence the insensible water loss. In one patient carefully studied by Newburgh for daily insensible water loss with calorimeter checks, excellent values were obtained with good correlation between the 2 methods for 4 of the 5 days. On the fifth day the young patient was visited by his fiancée and his insensible water loss on this day almost doubled the amount determined for the previous 4-day period. However, Newburgh himself was able to apply the method to ambulatory subjects and found close agreement with periodic calorimeter checks.

The dilution studies using D_2O and $S^{35}O_4$ are subject to errors also, and a conservative estimate of the error in such determinations in our hands is about 5 per cent.

The apparent discrepancy in the $S^{35}O_4$ space measurements require further investigation because of the paucity of information on the use of this isotope in surgical patients. The limitation of this method in patients who are subjected to trauma have not been defined.

SUMMARY

A modification of the Newburgh formula for the calculation of changes in body fat has been applied to 5 patients who were subjected to operations of varying degrees of severity. This method has been compared with 3 other indirect methods for fat change which were based upon isotope dilution. The results of the space studies in this report suggest that an extension of those methods would provide a means of determining water shifts between the intracellular and extracellular compartments. Further refinement of these methods should provide more accurate determination of body fat changes in surgical patients.

ABSTRACTS OF CASES

CASE 1.—E. A. (NYH 263940), a 66-year-old white woman, was admitted to the New York Hospital for the fourth time on Oct. 3, 1957, with the chief complaint of epigastric pain of 7 weeks' duration associated with nausea, vomiting, tarry stools, and a weight loss of about 4 Kg. in 2 weeks. Because of an anemia her local physician had given her 2 transfusions. Past history, systemic inventory, and physical examination were noncontributory. Laboratory results were unremarkable except for a hematocrit of 34 per cent and a gastrointestinal series showing a filling defect in the cardia of the stomach. Five days preoperatively she was transferred to the Surgical Metabolism Unit. Initial weight was 67.4 Kg. She received oral feedings giving an average total caloric intake of 27 calories per kilogram per day and an average nitrogen intake of 0.24 Gm. per kilogram per day. She was given 50 mg. Nilevar intramuscularly for the 3 days preceding operation. On Oct. 11, 1957, a leiomyoma of the gastric cardia was removed by wedge resection and the postoperative course was unremarkable. The average caloric intake following operation by both oral and parenteral routes was 10.5 calories per kilogram per day and the average nitrogen intake was 0.09 Gm. per kilogram per day. Nilevar administration was continued for one week following operation. She was discharged on a 6-feeding gastric diet 9 days postoperatively.

CASE 2.—A. H. (NYH 512342), a 57-year-old white woman, was admitted for the first time on Oct. 2, 1957, complaining of epigastric pain of 3 weeks' duration. The initial episode had been one of great severity and was associated with nausea and vomiting. The past history was noncontributory. Physical examination revealed a thin, nervous, middle-aged white woman complaining of abdominal pain. There was moderate mid-epigastric tenderness. Laboratory studies revealed the presence of free acid in a fasting gastric specimen and a 4+ stool guiac. Gastrointestinal series showed a large gastric ulcer with penetration into the pancreas. After a period of nasogastric suction the patient's pain subsided and she was transferred to the Metabolism Unit for a period of 11 days prior to operation. Initial body weight was 58.6 Kg. Oral feedings were supplemented by intravenous Lipomul and 5 per cent Aminosol in 5 per cent dextrose in water to give an average caloric intake of 49.5 calories per kilogram per day and an average nitrogen intake of 0.26 Gm. per kilogram per day. She received Nilevar 50 mg. intramuscularly for 3 days preoperatively and 6 days postoperatively. On Oct. 15, 1957, partial gastrectomy was carried out for a gastric ulcer perforating into the pancreas. The postoperative course was uneventful except for a transient episode of

jaundice secondary to Nilevar administration. Intravenous fat and nitrogen supplemented the oral intake to supply an average of 30 calories per kilogram per day and 0.27 Gm. of nitrogen per kilogram per day for 10 days. She was discharged on a 6-feeding gastric diet on the tenth day following surgery.

CASE 3.—Z. K. (NYH 784982), a 44-year-old white woman, was admitted for the first time to The New York Hospital on Jan. 12, 1958, with a history of epigastric pain and weight loss of 9 Kg. for the previous 18 months. Past history, systemic inventory, and physical examination were unremarkable. Laboratory studies were unrevealing except for an upper gastrointestinal series which revealed an antral filling defect. In the Metabolism Unit she received an oral intake affording 38 calories per kilogram per day and 0.27 Gm. of nitrogen per kilogram per day for 6 days preoperatively. On Jan. 20, 1958, the patient underwent subtotal gastrectomy for an antral carcinoma. The intial postoperative course was unremarkable. The patient received an average of 20 calories per kilogram per day and 0.14 Gm. of nitrogen per kilogram per day via parenteral and oral routes for the 11 days after surgery. After the termination of the study, the patient was transferred to the medical service.

CASE 4.—F. M. (NYH 776151), a 64-year-old Negro woman, was admitted to The New York Hospital for the first time on Oct. 25, 1957, with a 9-month history of epigastric pain, nausea, and vomiting and a 23 Kg. weight loss. Past history and systemic inventory were noncontributory. Physical examination revealed a markedly emaciated elderly Negro woman with moderate upper abdominal tenderness. The gastrointestinal series revealed a carcinoma of the gastric cardia. She received an oral intake of formula affording 24 calories per kilogram per day and 0.17 Gm. of nitrogen per kilogram per day for 7 days preoperatively. Weight on admission was 65.6 Kg. The bowel was perpared with neomycin. On Nov. 6, 1957, a palliative total gastrectomy with jejunal interposition was performed for a large tumor of the gastric cardia with extensive nodal metastases. In the postoperative period the patient was febrile; temperature averaged 38.5° C. rectally. She received an average of 17 calories per kilogram per day and 0.12 Gm. of nitrogen per kilogram per day until discharge on the thirteenth day.

CASE 5.—C. C. (NYH 163644), a 66-year-old white woman, was admitted to The New York Hospital for the first time on Oct. 23, 1957, with a history of epigastric pain, anorexia, and a 0.75 Kg. weight loss of 6 months' duration. The patient had suffered from a duodenal ulcer over the past 20 years. System inventory and physical examination were negative. Laboratory studies were negative except for a gastrointestinal series showing a carcinoma of the gastric antrum. She received 28 calories per kilogram per day and 0.20 Gm. of nitrogen per kilogram per day by oral feedings for 7 days prior to operation. The initial weight was 56.4 Kg. The bowel was prepared with neomycin. At operation on Nov. 1, 1957, a total gastrectomy with jejunal interposition and splenectomy was carried out for an antral carcinoma with nodal metastases. Postoperatively she received penicillin and dihydrostreptomycin. Her course was complicated by the occurrence of staphylococcal enterocolitis recognized on the third postoperative day. Because of this she was maintained on ACTH from the third to the fourteenth day after operation. Erythromycin and Albamycin antibiotics, whole blood, plasma, and electrolyte solutions were also administered. She remained in critical condition for a week following the onset of the infection and then gradually improved until the time of discharge on the twenty-third postoperative day. From the ninth postoperative day, the patient received an average of 28 calories per kilogram per day and 0.20 Gm. of nitrogen per kilogram per day.

We wish to express our appreciation for the help and technical assistance of Mr. Alan Stevenson, Mrs. Margaret Miller, Miss Iliria Delvina, Mr. Rene Petigny, and Mrs. Sema Lerner. Our dietitian, Miss Barbara Skaug, is responsible for the formula fed the patients and also acted as assistant for the myriad of calculations necessary.

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(For discussion, see page 146.)

RE-EVALUATION OF THE ROLE OF THE ADRENAL AND OTHER FACTORS IN THE METABOLIC RESPONSE TO SURGERY

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INTRODUCTION

DURING the past several decades numerous studies have shown that following a thermal burn or fracture patients exhibit a retention of water and sodium and an increased renal excretion of potassium and nitrogen. It has been evident that the degree and duration of water and sodium retention and of potassium and nitrogen wasting is largely dependent upon the severity of the trauma and occurrence of complications (blood loss, fever, dehydration, and so forth). It has also been found that the drop in plasma volume and red cell mass and the occurrence of malnutrition could be minimized and in some instances prevented by giving essential nutrients to these patients. More recently postoperative studies of patients undergoing elective surgery have shown that the same metabolic alterations occur but usually they are of a lesser magnitude. While the nutritional and endocrinologic alterations following elective surgery are similar to those noted after a thermal burn, it has become a common practice for many physicians to withhold water, sodium, potassium, and nitrogen during the early postoperative period. This method of treatment has been adopted because of the widespread belief that the overactivity of the pituitary-adrenal axis causes an obligatory postoperative metabolic response and that the administration of water, sodium, potassium, and nitrogen might be harmful or that these substances would not be properly utilized.

We believe that during the early postoperative period surgical patients should be given adequate fluid and nutrients in order to aid in the restoration of a normal physiologic state. We also support the view that the primary effect or purpose of the endocrine response following trauma is to aid in the restoration and maintenance of homeostasis.

The purpose of these studies was to obtain further information concerning the effects of giving water and nutrients during the early postoperative period when a state of overactivity of the pituitary-adrenal axis exists.

This work was supported in part by grants from the National Institutes of Health, U. S. Public Health Service A-760(C6), Baxter Laboratories, Morton Grove, Ill., Mead Johnson and Company, Evansville, Ind., and the John A. Hartford Foundation.

Read at the Annual Meeting of the Society of University Surgeons, Boston, Mass., Feb. 13-15, 1958.

METHODS AND RESULTS

Pre- and postoperative balance studies and daily determinations of weight and urinary 17-hydroxycorticoids have been carried out on 2 groups of male and female patients, one group undergoing cholecystectomy and the other subtotal gastrectomy. These studies were conducted on the Metabolic Division of the University Hospitals of Cleveland. All nutrients which were taken orally or given intravenously as well as all excreta were analyzed for nitrogen, sodium, and potassium according to previously described methods.[1] Accurate collections were made of all output including urine, feces, sputum, and gastrointestinal drainage. The daily urinary 17-hydroxycorticoid excretion was determined according to the method of Glenn and Nelson.[2] Each patient was weighed on an accurate scale daily at 6:30 A.M. after voiding and having refrained from the taking of food or fluid for at least 6 hours. Nutritionally complete diets were planned according to the patient's own selections from a variety of previously analyzed foods and were kept constant during the preoperative period when the patient was on an oral intake. The first group (patients undergoing cholecystectomy) were maintained on intravenous alimentation during the 3-day preoperative control period and all of the patients were given nutrients solely by the intravenous route for a minimum of 3 days postoperatively. Fluids were administered intravenously to the patients undergoing cholecystectomy at a rate of approximately 500 ml. per hour. The patients undergoing gastrectomy were given their fluids at a rate which would not exceed 0.5 to 0.6 Gm. of hexose per kilogram of body weight per hour. Ambulation was started on the day of operation.

Balance data are shown in the following manner. Intake is plotted from the base or zero line upward and the output (the sum of urine, feces, sputum, and gastrointestinal drainage) is plotted downward from the top of the intake column. A black area below the zero line represents negative balance while a white hatched area above the zero line represents positive balance. The days when the intake was given intravenously are indicated by the black hatched area and the days when the intake was oral by the white area in the graph which shows the caloric intake. The numbers below the balance columns represent the cumulative gain or deficit of the substance during a 3-day period except for one 2-day control period (N. R., Fig. 2). Blood transfusions were given in amounts necessary to replace operative losses and are not shown on these charts nor is any allowance made for insensible losses of water and electrolytes. The vertical broken line indicates the day of operation. (A half circle indicates that the determination was not done for that day.)

Fig. 1 shows the data obtained from the study of 3 women (B. J., H. W., and D. W) and 2 men (M. M. and A. N.) who underwent elective cholecystectomy for chronic gallbladder disease. These patients were all in a normal nutritional state at the time the studies were undertaken and their postoperative courses were uneventful. Preoperatively each patient was given a nutritionally complete oral diet for 1 or 2 days. This was followed by a 3-day control period during which their only intake was daily intravenous infusions of 5 per cent dextrose in water. The amount given daily was kept constant for each individual but varied slightly from patient to patient. After the control period, there was a 3- or 4-day repletion period during which time the patients ate the same diet they had taken at the beginning of the study. This was done in order to have the patient in a comparable nutritional state at the beginning of both the control and postoperative periods. Cholecystectomy was performed under general anesthesia on the day following the oral repletion period. On the day of operation and for the next 2 days, the patients were given intravenous infusions of the same volume of 5 per cent dextrose in water daily as they had received during the control period. During both study periods these 5 patients exhibited a positive water balance if one excludes the insensible fluid loss. However, if insensible losses are considered, all of the patients with the exception of A. N. exhibited water deficits. All the patients showed deficits of sodium, potassium, and nitrogen during both the control and postoperative periods since there was no intake of these substances. Although there is variation from patient to patient the cumulative 3-day control and

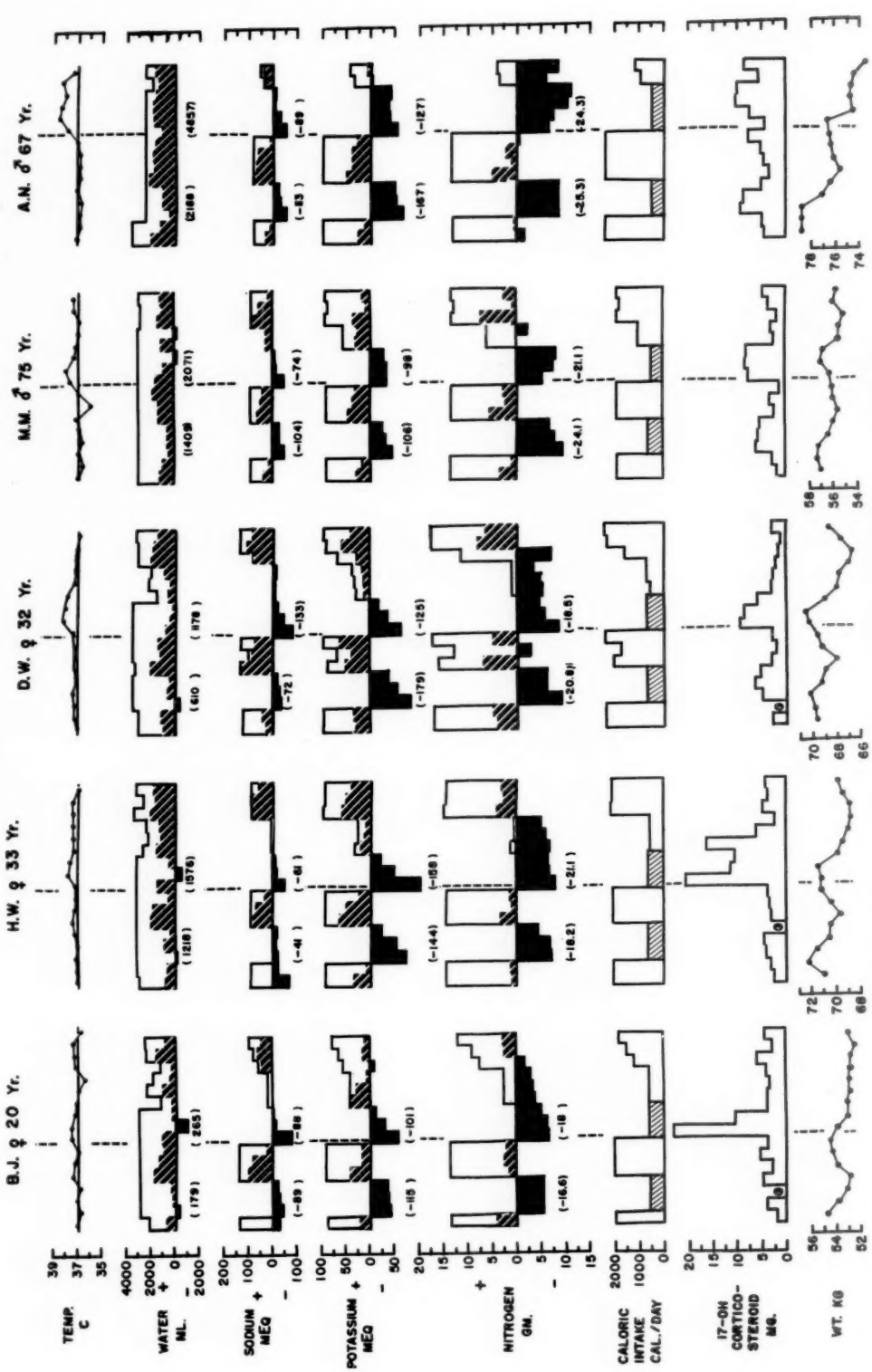


Fig. 1.—Metabolic balance charts of 3 women and 2 men who underwent cholecystectomy. Each patient was maintained on the same daily intravenous regimen of 5 per cent dextrose in water for a 3-day control period, on the day of operation, and first 2 postoperative days.

postoperative deficits of sodium, potassium, and nitrogen of each patient are comparable. The 2 men and one woman (B. J.) had slightly larger sodium deficits during the control studies than during the postoperative periods, while the other 2 women (H. W. and D. W.) had larger deficits during the postoperative period. All of the patients except H. W. had slightly greater deficits of potassium during the 3-day control study than during the postoperative period. During both the control and postoperative periods, all the patients showed a gradual diminution in the magnitude of their daily deficits of sodium and potassium. Postoperatively the 3 women had nitrogen deficits which averaged 6.4 Gm. per day while an average daily nitrogen deficit of 7.6 Gm. was shown by the 2 men. Similar weight losses occurred during the pre- and postoperative 3-day periods of intravenous alimentation.

Fig. 2 shows the data obtained from a group of 4 women (L. B., M. O., M. J., and N. R.) who also had an elective cholecystectomy and were studied in the same manner as the preceding group of patients. However, during the 3-day control and postoperative periods they were given daily intravenous infusions of 5 per cent dextrose in water supplemented with 90 mEq. of sodium and 80 mEq. of potassium. One woman in this group (N. R.) had a control period of only 2 days and an oral repletion period of only 1 day. When estimated insensible water losses are considered L. B. and M. J. exhibit moderate 3-day deficits during both the control and postoperative periods. Patients M. O. and N. R. show essentially the same findings during the control periods but were approximately in equilibrium during the postoperative period. These 4 women showed sodium deficits during the control period. However, during the postoperative period, L. B. and M. J. had sodium deficits while M. O. and N. R. were in positive sodium balance. During the control period L. B. had a significant potassium deficit while the other 3 patients fluctuated slightly from equilibrium. During the 3-day postoperative period L. B., M. O., and M. J. exhibited significant potassium deficits while N. R. was essentially in equilibrium. The 4 patients were in negative nitrogen balance during both the control and postoperative periods. L. B., whose postoperative course was complicated by a wound abscess and fever, exhibited a cumulative 3-day postoperative nitrogen deficit of 33.3 Gm. and a potassium deficit of 160 mEq. These deficits which are greater than those of the other patients are attributed to the presence of infection. Considerably smaller nitrogen deficits were noted during the control period in M. O. and M. J. than any of the other 7 patients in the first 2 groups or in normal volunteers on a nitrogen free 500 calorie oral diet. We cannot account for these low values during the control periods. However, the postoperative deficits of M. O. and M. J. were comparable to the control and postoperative deficits of N. R. and the patients in the first group. It is worth noting that the postoperative 3-day cumulative nitrogen deficits of the 6 women in groups one and two whose postoperative courses were uncomplicated ranged between 15.7 and 20.7 Gm. with an average of 17.4 Gm. or an average daily deficit of 5.8 Gm. Three women (L. B., M. O., and M. J.) had weight losses during the control and postoperative periods comparable to those exhibited by the first group of patients while N. R. showed a slight gain of weight postoperatively.

The data from the study of 4 women (S. M., E. T., H. R., and E. H.) who also underwent elective cholecystectomy are shown in Fig. 3. This group of patients was studied in the same manner as the 2 preceding groups. However, during the 3-day control and postoperative periods, these patients were given daily intravenous infusions of a 5 per cent protein hydrolysate in 10 per cent fructose* supplemented with sodium and potassium to provide a total daily intake of approximately 90 mEq. of sodium and 80 mEq. of potassium. These patients were also given additional amounts of 10 per cent fructose in water in order to supplement their fluid and caloric intakes. If insensible water losses are considered, these 4 patients preoperatively would exhibit deficits and postoperatively would vary between a slight deficit and equilibrium. The 4 patients were all in negative sodium balance during the control period. Three of them (S. M., H. R., and E. H.) were also in

*Mead Johnson and Company, Evansville, Ind., kindly supplied these solutions.

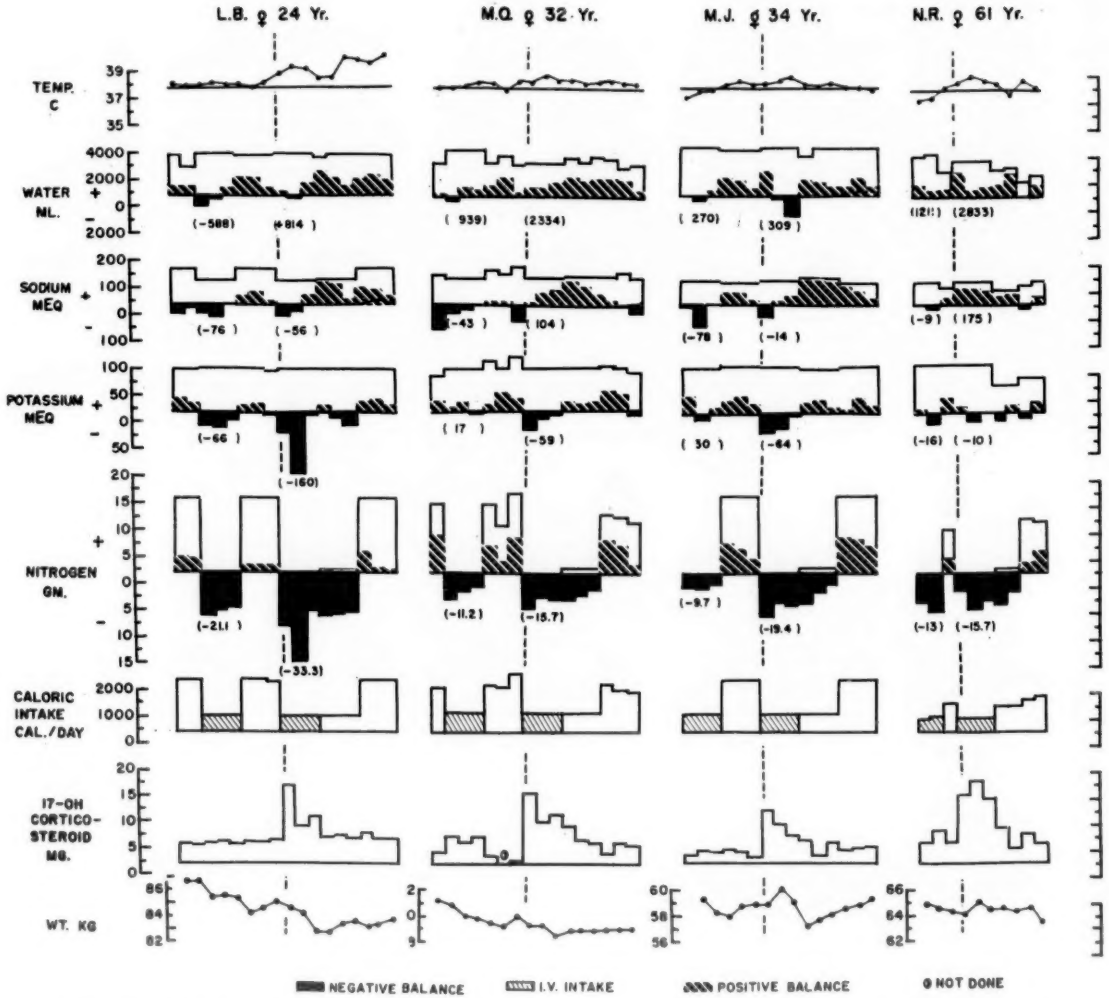
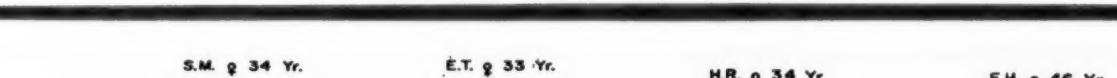


Fig. 2.—Metabolic balance charts of 4 women who underwent cholecystectomy. Each patient was maintained on the same daily intravenous regimen of 5 per cent dextrose in water supplemented with approximately 90 mEq. of sodium and 80 mEq. of potassium for a 3-day control period, on the day of operation, and first 2 postoperative days.



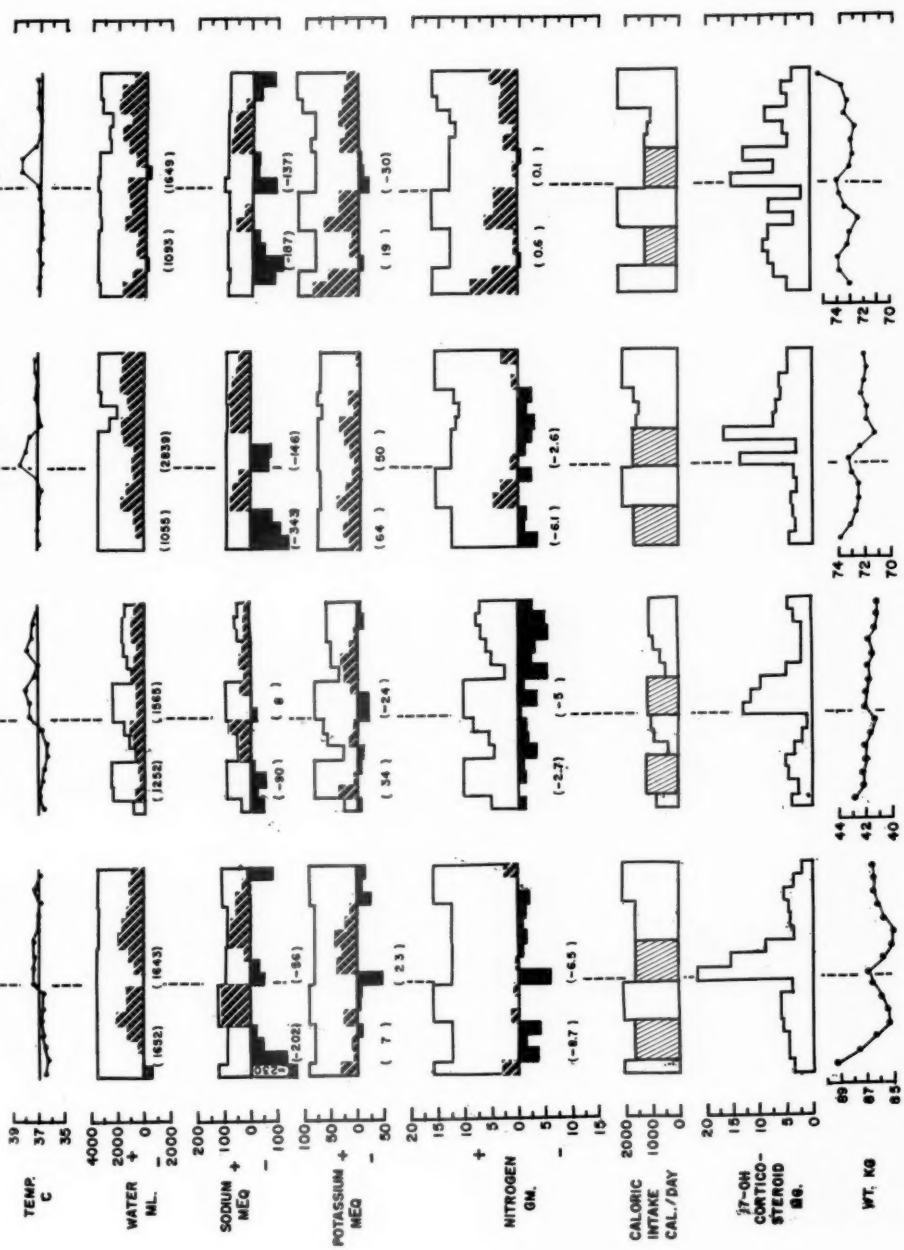


Fig. 3.—Metabolic balance charts of 4 women who underwent cholecystectomy. Each patient was maintained on the same daily intravenous regimen of a 5 per cent protein hydrolysate in 10 per cent fructose containing approximately 90 mEq. of sodium and 80 mEq. of potassium for a 3-day control period, on the day of operation, and first 2 postoperative days.

negative balance postoperatively but to a lesser degree, while the fourth (E. T.) was essentially in equilibrium. The potassium balances during the two 3-day study periods were not significantly different in the case of any individual patient. Postoperatively 2 patients showed slight retention and the others slight deficits of potassium. Each of the 4 patients had comparable control and postoperative cumulative nitrogen deficits which were considerably less than the deficits exhibited by the preceding 2 groups who were maintained on nitrogen-free, 500 to 700 calorie intravenous regimens. Three of the 4 patients (S. M., H. R., and E. T.) had intakes of 0.14 to 0.17 Gm. of nitrogen and 19 to 23 calories per kilogram of body weight daily. These patients showed some weight loss during both 3-day periods. These nitrogen and caloric intakes are considerably less than that recommended by Rhoads.[3] In contrast, E. T., who showed minimal weight changes during the control and postoperative periods, had an intake of 0.3 Gm. of nitrogen and 30 calories per kilogram of body weight.

The normal range for 24-hour urinary excretion of 17-hydroxycorticoids determined according to the method of Glenn and Nelson[2] is 4.5 to 7.0 mg. for men and 3.5 to 6.0 mg. for women. The 13 patients whose studies are included in Figs. 1, 2, and 3 all had 24-hour 17-hydroxycorticoid excretions in the urine within the normal range during their control and repletion periods. On the day of operation, the 13 women exhibited urinary 17-hydroxycorticoids 2 to 5 times greater than their control values. Over the next 2 days there was a gradual decrease toward normal values. The 2 men demonstrated only slight but not significant increases in their urinary corticoids postoperatively. Neither had any clinical nor laboratory evidence of adrenal insufficiency.

The data obtained from the studies of 40 male and female patients during the first week following a subtotal gastrectomy can be summarized as follows.

1. The urine volume was less on the day of operation than that noted on preoperative patients and volunteers who were maintained on a comparable regimen. In almost every instance there was an increase in the urine volume on the day following the operation. Smaller urine volumes were noted to occur in patients who were undernourished or in those who had complications.

2. Intakes of 80 to 100 mEq. of sodium resulted in slight positive or negative balance. When greater amounts of sodium were given or when malnutrition, complications, cardiac failure, or other disorders in which sodium is not excreted in a normal fashion occurred, a positive sodium balance resulted.

3. When patients were maintained on oral or intravenous regimens which were deficient in sodium, potassium, and nitrogen, deficits of these substances occurred. It was noted that greater deficits of potassium and nitrogen occurred in men than in women when they were maintained on comparable regimens. These deficits were of a lesser magnitude in the chronically diseased or malnourished patients and were increased following complications (fever, infection, hemorrhage, dehydration, and so forth).

4. When from 20 to 100 mEq. of potassium was given daily and in the absence of complications, the balance was primarily dependent upon the amount of potassium given. The patients whose daily intakes of potassium were 60 mEq. or less usually showed a negative potassium balance while patients given between 60 and 100 mEq. of potassium per day showed only slight deviation from equilibrium. Patients who exhibited a fairly marked protein deficit or prolonged negative nitrogen balance usually exhibited a potassium deficit.

5. When good nutritional intakes were provided and no complications occurred there were minimal changes in weight and nitrogen balance.

6. There was no correlation of the water, sodium, potassium, and nitrogen balances and changes in weight with the urinary 17-hydroxycorticoid excretion. Several of the patients exhibited no significant increase in their 17-hydroxycorticoid excretion following operation. The balance data obtained from the study of these patients were comparable to those obtained from the study of the other patients.

7. There was often a lack of correlation between the weight changes and nitrogen balance because of gains or losses of water and sodium.

DISCUSSION

The postoperative metabolic alterations of patients reported in the present study were primarily influenced by the previous nutritional state, age, and sex of the patient, nutritional regimen employed, and the development of postoperative complications. Somewhat greater nitrogen deficits occurred following a subtotal gastrectomy than after a cholecystectomy. Although the urinary 17-hydroxycorticoids, with a few exceptions, increased postoperatively there was no correlation with either water, sodium, potassium, and nitrogen balances or the magnitude of the operation. The data obtained from balance studies per se should not be used as the sole guide in determining therapy. A positive sodium and water balance will be found in patients with either cardiac failure or those who have a sequestration of fluids because of peritonitis. The salt and water intake should be limited in the former case, while the latter patient should be given adequate amounts of fluid and electrolytes in order to maintain an effective circulating blood volume.

The lack of a consistent pattern of postoperative metabolic alterations in the patients reported in the present study supports the belief that the biochemical and physiologic changes associated with operative trauma such as development of a "third space," hemorrhage, abnormal extrarenal losses, and so forth cause an increase in the activity of the pituitary-adrenal axis. Since the primary effect of the hormones elaborated by these endocrine glands is the maintenance of homeostasis, adequate amounts of water, electrolytes, nitrogen, and calories should be given to the postoperative or posttraumatic patient to aid this endocrine response in the restoration and maintenance of a normal physiologic state.

CONCLUSIONS

Metabolic studies were conducted in 53 male and female patients undergoing a cholecystectomy or partial gastrectomy. The following conclusions were derived from the data obtained from these studies:

1. The postoperative metabolic alterations are influenced by the preoperative nutritional status, therapy employed, sex, occurrence of complications, endocrine response, and the existence of other disorders.

2. Postoperatively there was no correlation between the endocrine activity as measured by the urinary excretion of 17-hydroxycorticoids and the water, sodium, potassium, or nitrogen balance in patients maintained on various nutritional regimens.

3. The postoperative patient should be given adequate amounts of fluids, electrolytes, vitamins, nitrogen, and calories to aid the endocrine response in the maintenance of homeostasis.

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Discussion

(Papers by Spencer, Neill, and Bahnson; Krieger, Abbott, Levey, and Holden)

DR. FRANCIS D. MOORE (Boston, Mass.).—I would like to divide my discussion into 3 parts: (1) compositional changes, (2) the meaning of magnitude in trauma, and (3) our current ideas on the adrenals.

It seems that many processes alter body composition in the direction of increased E.C.F., decreased I.C.F. and fat, with an increase in body water and sodium and a decrease in potassium. This is a mass response to many forms of disease. It includes x-ray treatment, total body irradiations, starvation, trauma, sepsis, and the presence of malignancy in the body.

It is interesting that many endocrine stimuli also tend to do the same thing to a greater or lesser degree, including the secretions of the anterior and posterior pituitary and both the adrenal medulla and cortex.

We have come to feel that the best way to express the magnitude of injury is the sum total of the biological components involved and, for most surgical patients, these include the actual cross-sectional tissue injury, shock or volume reduction, anesthesia which is a tremendous variable, desalting and water loss, which are stimuli, medication, the presence of necrotic tissue, which is an additional factor, and infection.

The more profound the magnitude of the trauma, the greater the sum total of these biological components and the greater is the tendency to exhibit decompositional change. The less the extent of the trauma, the less the compositional change and the easier it is to reverse it or obviate it with hormones or therapy of a nutritional type.

Dr. Krieger and his group have re-emphasized that starvation plays a very important role in body compositional change, sex factors, age, preoperative starvation, preoperative state, and so on. But it should be emphasized that their group of patients is a fairly homogeneous group, having a rather homogeneous type clean, elective, civilian, soft tissue surgery, and we have not been able to get the same results using patients having accidental trauma, such as compound fractures of the femur or major multivisceral operations with shock. Then the traumatic components begin to add up and it gets to be harder and harder to wipe out the compositional change by what you give.

We, too, have been interested in what the adrenals have to do with all this and seem to have come to much the same conclusion as Drs. Krieger and Holden. Surely, the last word has not been stated, but decreased effective volume is a major stimulus to the production of 17-hydroxyaldosterone and A.D.H. The work of Barker at Bethesda is classical in this regard, showing that 500 c.c. of blood taken out quickly produces a maximal secretion of aldosterone and that, when it is given back an hour or so later, the adrenal answers immediately and stops putting out the material.

It is interesting that the absence of 17-hydroxyies, aldo, and A.D.H. lead, to one degree or another, to hypotension and shock, and it certainly seems to us increasingly that decreased effective volume, flow, perfusion, oxygenation, some parameter of cardiac output is a primary adrenal stimulus in most surgical practice and the metabolic changes may be related and, if related, may only be a by-product.

DR. HENRY THOMAS RANDALL (New York, N. Y.).—This problem of indirect measurement of fat loss is probably insoluble, at least by any present known technique. Dr. Cornell's illustration of the variability of 4 simultaneous methods of calculation well illustrates this.

Yet, there is available to us in these studies a method which we have not used except for one paper from Dr. Moore's group that I recall, and that is to go back to some of the basic calorimetric studies that have been done before by some of our colleagues in medicine. Were it possible, for example, to maintain a surgical patient under complete caloric control or, more particularly, perhaps, just simply to measure the CO_2 output over a given period, 24 hours or so, with nitrogen balance, it would then be possible to calculate absolutely the patient's oxygen consumption or CO_2 output, caloric use in the course of 24 hours, and, therefore, fat loss.

There is little doubt that patients who undergo trauma use far more calorically than we think they do. Some of these data would lead us to that conclusion and that the caloric losses are very high.

Some years ago, Dr. Sidney Werner, Dave Habif, John Lockwood, and I did similar studies with patients with cholecystectomy and there is no doubt that this relatively benign surgical procedure is capable of being compensated for completely intravenously. We found that we could maintain patients in isocaloric and isonitrogen balance with about 35 calories per kilogram per day intake.

There is no doubt, however, also, that in major trauma and large surgical procedures the demands go up so high that, unless there is a good fat preparation today which does not have any side effects, we are still not capable of maintaining complete nutrition. Whether or not it is wise to attempt in the elective, clean, civilian case to maintain an isocaloric and isonitrogen intake is still a matter for speculation. It is difficult to do in metabolic units carefully controlled. Practically speaking, in a ward or in an operating room, with a turnover of patients in civilian practice, it is probably not worthwhile, because most patients are perfectly capable of taking the insult and the complications of trying to keep up with them are so vast that our own abilities and those of our house staffs are better put to other uses.

However, in the major case of massive trauma in the patient who is markedly depleted it is no doubt worthwhile and indeed lifesaving to use all of the facilities at our command as well as our knowledge of these effects to attempt to maintain as much nutrition as well as electrolyte balance as we know how.

DR. ROBERT E. L. BERRY (Ann Arbor, Mich.).—I know that some of you remember Mr. Andrew Wilkinson, the Scottish surgeon who made a trip to this country about a year ago with his very interesting and unique observations on the postoperative use of fluids, which essentially consisted of using no fluids at all. After he had dropped that bombshell in our laps, he said, "And what do you think about that?" I said, "I have come to two conclusions. Number one, indeed, the Scots are a very hearty people and, two, even in the tradition of your nation, that is carrying things a little bit too far."

In exploring some of the ramifications of the internal movement of electrolytes, I would like to demonstrate to you what happens to patients who do not receive any fluids in the postoperative period.

(Slide.) This is a 21-year-old boy. The only fluid he received for 72 hours after operation—that is, water—was 250 ml. of 5 per cent glucose and water which contained 0.2 of 1 per cent of sodium chloride. Subsequent to that, he got absolutely nothing. It is extremely difficult to keep people on no fluids in the immediate postoperative period and it is not recommended for routine use. It is only recommended as an investigational tool.

We were using Na^{22} which has a half-life of 2.8 years, and a biological life of approximately 18 days. When we gave the routine, conventional type of fluids, we would see an increase of the sodium space. The exchangeable sodium measured in milliequivalents per kilogram, based upon percentage change in conventional fluids will increase as much as 25 to 30 per cent.

When fluids are withheld the increase is in the so-called space. The increase in the changeable sodium is almost small enough to fall into the range of laboratory error. The same thing applies to the serum sodium concentration and the osmolarity. There the changes are so little that actually they do fall into the range of laboratory error.

After this man had been on nothing at all for 72 hours, almost at the eightieth hour we gave him 2,000 ml. of 5 per cent glucose and water containing about 90 milliequivalents of sodium. See the sharp increase in the exchangeable sodium as well as a rise in sodium space. Certainly, many of the space changes that we see are triggered by the fluids that we give, because other cases which we have similarly resemble these curves when no fluids are given at all for 72 consecutive hours postoperatively. What, then, is

the source of the increase in exchangeable sodium under the circumstances? I am sure that our data did not give us the answer. It did seem that perhaps trauma was making available sodium from somewhere that ordinarily is not exchangeable unless trauma is superimposed, or perhaps there are changes in the flux rate of sodium across the cell membranes.

DR. BEAL (Closing).—Dr. Randall has certainly struck upon the crucial point which is really the point of our paper. The energy exchange is the problem toward which we are working in trying to assess fat changes in the postoperative period. While direct calorimetry is honestly the variable end, it also seems to offer extreme difficulties and, until such time as someone has the ability and can afford to accomplish this, we are stuck with indirect methods. This is indeed a very important point in the future of fat determination.

ANNOUNCEMENT

The Tenth Annual Postgraduate Assembly in Endocrinology and Metabolism of the Endocrine Society will take place from Sept. 29 to Oct. 3, 1958, at the State University of New York Upstate Medical Center in Syracuse, N. Y. Of special interest to surgeons will be a panel on oncology and endocrinology. The course will feature 29 specialists in the field of endocrinology and metabolism from all over the country. Further information can be obtained by writing to Dr. William A. Harris, Dean of Postgraduate Education, State University of New York Upstate Medical Center, 766 Irving Ave., Syracuse 10, N. Y. Registration is limited to 100.

THE FAVORABLE EFFECT OF PYRIDOXINE DEFICIENCY ON SKIN HOMOGRAFT SURVIVAL

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IN RECENT years, extensive evidence has been presented which would ascribe the mechanism of homograft failure to an actively acquired immunity by the host to the graft. The microscopic picture characterized by vascular thrombosis, inflammatory reaction with lymphocytic infiltration, and subsequent necrosis after an initial period of graft survival[17, 38, 43]; the interval of time between grafting and beginning rejection comparable to that required for the body to build up substantial antibody titers against the more common foreign antigens[5]; a more rapid rejection of a second homograft from the same donor to the same host in which a prior graft was established (''second set phenomenon'')[22, 42]; the relationship of the size of the graft to its length of survival[24, 36, 42]; and Bollag's[11] unconfirmed report of the detection of individual-specific antibodies to homografts by means of a turbidity test would all implicate an antigen-antibody response as the prime mechanism for homotransplant failure. For a more detailed analysis and further evidence of the immune concept of rejection, reference is made to the published transactions of the several conferences on homotransplantation[16, 45, 46] and recent review articles.[8, 12, 14, 17, 21, 28]

Based upon this concept, efforts, both experimental and clinical, have been directed toward either altering the transplant to enhance its acceptance by the recipient or interfering in some fashion with the reaction of the host. Concerning the former, freezing,[4] heating,[5] irradiation,[24, 31] and in vitro desensitization[19, 52] have all been reported. To date there is no reason for optimism with this approach to the problem.

Much effort has been directed toward altering the host response. The local and systemic use of cortisone,[9, 10, 13, 33] ACTH,[34, 41, 48] total body irradiation,[25, 31, 40] nitrogen mustard,[37] antihistamine drugs,[17, 30] salicylates,[17] reticuloendothelial blockade with trypan blue and thorium dioxide,[49] splenectomy,[35, 54] and anticoagulants[18] have all been used toward this end. For the most part, prolongation of homograft survival has resulted experimentally from only a few,

Aided by U. S. Public Health Service Grants C-3731 and RG-4624 and the Allegheny County Chapter of the United Fund.

Read at the Annual Meeting of the Society of University Surgeons, Boston, Mass., Feb. 13-15, 1958.

*John and Mary Markle Scholar.

and, of these, their clinical use would either seem highly impractical (that is, total body irradiation and nitrogen mustard) or they have already been shown to be of little value (such as, cortisone[27, 53]).

The ability of Billingham, Brent, and Medawar[7] to induce an "acquired tolerance" or a ". . . state of specific non-reactivity towards a substance that is normally antigenic—a non-reactivity, moreover, that is due to a primary failure of the immunological response"[6] has formed the basis of a new immunologic principle, the ramifications of which are not fully known. The findings of Woodruff and Simpson[57] that tolerance could be induced in rats by injection of cells shortly after birth in contrast to the embryonic injections of Billingham, and coworkers[7] suggested to them that a similar approach be tried in newborn infants. A preliminary report[55] would suggest that perhaps a state of partial tolerance had been induced, but it is recommended by them that, because of a possible danger to infants, intravenous injection of homologous cells should be avoided.

In 1946 it was demonstrated by Stoerk and Eisen[50] that reduced serum antibody concentrations occurred in pyridoxine deficiency. Axelrod and associates[1] and Carter and Axelrod[15] confirmed this finding and, in addition, noted a similar severe impairment of antibody response to human red blood cells as antigenic stimuli in pantothenic acid deficiency and moderate impairment in riboflavin, thiamin, and biotin-deficient rats. When purified diphtheria toxoid was used[44] as the antigen, essentially the same impairment of antibody response was noted.

On the basis of these findings, it was deemed worthwhile to observe the fate of skin homografts in rats made deficient in one of the afore-mentioned vitamins prior to grafting. Pyridoxine deficiency was chosen for this study because of its most consistent and marked effect upon antibody production. Encouraged by the findings and with a view toward clinical application, it was felt that greater practicability might be in store if similar results could be obtained with a specific-vitamin antagonist—in this case, desoxypyridoxine.

It is thus the purpose of this paper to present our findings regarding skin homografts in rats (1) made vitamin B_6 deficient by dietary means prior to grafting and (2) in rats treated subsequent to grafting with desoxypyridoxine. These studies were begun in the spring of 1955. For the sake of brevity, desoxypyridoxine will be designated as DB_6.

METHODS AND MATERIALS

Animals.—Data reported in this study was obtained from 1,529 male, weanling, littermate identified Wistar* and Long-Evans† rats, weighing between 55 and 65 Gm. upon arrival at the laboratory. All animals were weighed initially and once each week thereafter. Growth curves were plotted utilizing the average weight of the animals. They were placed in individual cages and begun on one of 3 dietary regimens which were ad libitum-fed.

Diet.—All animals were fed a basal diet consisting of sucrose 56.76 per cent, vitamin-free casein 25 per cent, salt mixture (Jones and Foster) 4 per cent, cod liver oil 2 per cent, hydrogenated vegetable oil (Crisco) 10 per cent, corn oil (Mazola) 2 per cent, choline

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†Simonson & Pacific Coast Farms, Gilroy and Los Angeles, Calif.

chloride 0.2 per cent, i-inositol 0.03 per cent, 2 methyl-naphthoquinone 0.001 per cent and alpha-tocopherol acetate 0.01 per cent. In addition, each rat received daily one of 3 vitamin pills with his food, depending upon whether he was on a (1) control or normal (50γ) vitamin B_6 intake, (2) a low vitamin B_6 diet (10γ), or (3) a vitamin B_6 free diet. Pills were prepared from a vitamin mix containing 160 mg. thiamin hydrochloride, 240 mg. of riboflavin, 400 mg. niacin, 800 mg. calcium pantothenate, and either 200, 40, or 0 mg. of pyridoxine hydrochloride, to which was added biotin (40 mg. of biotin plus 10 Gm. of dextrine) and folic acid (40 mg. of folic acid plus 40 Gm. dextrine). To this homogenous mixture, ethanol (70 per cent was added, so that the right consistency necessary for the preparation of pills was obtained. From this quantity about 4,000 pills, each of approximately 1 grain, could be made. All were similar, except that the pyridoxine hydrochloride content was 50γ, 10γ, or was absent.

To accustom them to the pill, animals were put on a 3-day "training" period. The first day they received only the pellet; the second day if the pills were eaten they received a small amount of basal diet. By the third day they almost all became accustomed to eating it. Subsequently, it was placed on top of the basal diet in the food dish where it was eaten almost immediately. This, too, was the habit of deficient animals who might not consume the basal diet.

All control rats in the vitamin B_6 dietary deficiency experiments received the 50γ pill with their food, while animals to be made deficient received no vitamin B_6. This diet was fed for 3 to 4 weeks before operation. Following grafting, most of the deficient animals were maintained on the vitamin B_6 free diet for 3 to 4 weeks and then were transferred to a stock diet of Purina Chow Checkers. In early experiments many animals were maintained on the deficient diet until death. The control animals, continuing to receive 50γ vitamin B_6 after grafting, were placed on the stock diet at a time similar to that for the deficient rats. All animals in experiments in which DB_6 was used were fed the basal diet plus 10γ of vitamin B_6 for 3 to 4 weeks before operation. Following grafting, control rats were maintained on this diet. Animals receiving DB_6 were placed on a vitamin B_6 free diet for 15 days following grafting. During the first 10 days DB_6 was administered. Subsequently, they were fed a 10γ vitamin B_6 diet for 3 to 5 weeks, and this was followed by the regular laboratory stock diet.

Desoxypyridoxine (DB_6)*.—In experiments in which DB_6 was administered, it was given 0.5 mg./100 Gm. of body weight intraperitoneally daily for 10 days, with the first injection immediately following operation.

Operative Procedure.—Animals were closely clipped, anesthetized with ether, and operative sites were swabbed with 70 per cent alcohol. Squares of full-thickness skin, measuring 2 by 2 cm., were removed from the mid-dorsum of all rats. This area was found by planimetric measurement of the skin surface of sacrificed animals to represent 7 per cent of the body surface. The removed skin was pinned to a cork board and the undersurface was gently scraped free of all fat and connective tissue. The recipient bed, being that square area of the back from which the donor skin was taken, was prepared by removing the thin panniculus carnosus so that the applied skin graft lay directly on muscle. Grafts, either auto- or homo-, were applied in such a way that hair growth would occur in reverse fashion to that of the host animal. They were sewn in place by numerous interrupted silk sutures with a careful approximation of skin edges. No dressing was applied. In early experiments, back skin was used exclusively for both auto- and homografts. Because of the unsatisfactory number of successful autograft survivals (Table I*A*) and the length of time required for absolute determination of the state of the graft due to the heavy superficial crust which formed, even on ultimately excellent "takes," the use of dorsal skin was abandoned. Pieces of skin similar in size to those removed from the back were excised from the anterior abdominal wall for both auto- and homografts, and, after preparing them in a fashion similar

*Nutritional Biochemical Co., Cleveland, Ohio.

to that described for back skin, they were applied to the square area on the mid-dorsum of the back. The removed piece of back skin was discarded. Extreme care was exercised to prevent grafting of littermates when animals of the same strain served as donor and recipient.

Terminology and Classification.—Although aware of the criticism by some[23] and of the semantics involved,[29] the word "take" throughout this paper will be used to mean a successful skin graft at the particular time noted following grafting. The one rigid macroscopic criterion of graft "success" that has been used by us is growth of hair in the opposite direction from that of the host. The lack of ulceration, contraction, or scar was also taken into consideration.

Two types of "nontakes" were encountered. One was the obvious bloody ulceration and/or necrosis with contraction. The other was characterized by a heavy indurated scab which persisted for many days and which, upon removal, revealed a smooth glistening fibrous scar from which no hair grew and which, upon histologic examination, was found to be free of hair follicles and with a disturbed collagen. No separate consideration of these will be made; they will all be categorized as nontakes.

It soon became apparent that there were various degrees of takes and that it would be advantageous for more critical study to establish 3 arbitrary subgroups. A +2 take indicates profuse new hair growth over the entire graft. In some instances this graft may have contracted slightly from its original size. A +1 take represents a good take with new profuse hair growth over a portion of the graft, the remainder being fibrous tissue, crust, or an area of ulceration. This type of take was frequently encountered when the animal chewed away a portion of the graft, when a technical fault, such as stitches pulling out, occurred, or when a hematoma beneath a portion of the graft occurred. Occasionally, a graft was placed in this classification if it appeared to have taken in toto, but hair growth, although present throughout, was not as profuse as in the +2 graft or as minimal as in that group labeled a ± take. This latter classification (±) covers takes in which there were scattered wisps of new hair over the entire graft surface, which in itself exhibited little or no contraction.

During these experiments numerous animals were sacrificed at various times following grafting for routine histologic and special histochemical studies. Sections from all grafts that were questionable were studied microscopically. The details of these studies will be described in a subsequent report.

RESULTS

To facilitate presentation of data, the results obtained from 18 separate experiments have been collected into 4 major groups: (1) autografts, (2) control homografts, (3) homografts in animals made vitamin B_6 deficient by dietary means prior to grafting, and (4) homografts in rats receiving DB_6 subsequent to grafting.

TABLE I. FATE OF AUTOGRAFTS 30 DAYS AFTER GRAFTING

TYPE OF SKIN	DIET	NUMBER OF RATS	TAKES				NON-TAKES	PER CENT TAKES
			+2	+1	±	TOTAL NUMBER		
A. 311 Wistar								
Back	Control	69	4	20	7	31	38	45
Abdominal	Control	95	84	7	1	92	3	97
Back	Deficient	64	26	18	11	55	9	86
Abdominal	Deficient	35	29	6	0	29	0	100
Abdominal	Deficient + DB_6	48	40	8	0	48	0	100
B. 150 Long-Evans								
Abdominal	Control	85	71	10	0	81	4	91
Abdominal	Deficient	22	14	6	2	22	0	100
Abdominal	Deficient + DB_6	43	30	5	4	39	4	91

1. Autografts.—A summary of the fate of autografts 30 days after grafting in 461 Wistar and Long-Evans rats is presented in Table I, A and B. These may be considered as permanent survivors. Not once did successful autografts at 30 days, when subsequently observed for as long as 6 months, deteriorate.

Wistar: With back skin grafted to control animals, only 45 per cent were takes, and of these only three-fourths were of the +1 or +2 variety. When the thinner abdominal skin was grafted in similar animals, 97 per cent survived and they were almost 100 per cent excellent. Better results using back skin were obtained in autografts of deficient animals (86 per cent takes). But even this was not as good as when abdominal skin was applied to animals on a deficient diet or receiving DB_6, where 100 per cent survival occurred. There were only 3 failures in 178 Wistar animals, control or deficient, autografted with abdominal skin.

Long-Evans: Equally good results were found when abdominal skin was autografted in either control or deficient Long-Evans rats. In this group of 150 animals there were but 8 nontakes. Of the 142 takes, 95 per cent were of the excellent variety (+1 or +2).

2. Control Homografts.—Five hundred and fifty-three homografted rats served as controls for those either made vitamin B_6 deficient prior to grafting or receiving DB_6 subsequent to the operation. All animals were either on a 50γ or a 10γ vitamin B_6 diet. A complete summary of data collected from these rats as of 30 days after grafting may be found in Table II, *A* and *B*.

TABLE II. FATE OF HOMOGRAFTS 30 DAYS AFTER GRAFTING

DONOR	RECIPIENT	TYPE OF DONOR SKIN	NUMBER OF RATS	TAKES				NON-TAKES	PER CENT TAKES
				+2	+1	±	TOTAL NUMBER		
A. 333 Wistar rats on a control diet									
Wistar	Wistar	Back	78	0	8	4	12	66	15
Wistar	Wistar	Abdominal	53	11	3	2	16	37	30
Long-Evans	Wistar	Abdominal	202	4	2	2	8	194	4
B. 220 Long-Evans rats on a control diet									
Long-Evans	Long-Evans	Abdominal	27	1	0	0	1	26	4
Wistar	Long-Evans	Abdominal	193	5	8	1	14	179	7

Wistar recipient:

a. Twelve of 78 (15 per cent) of homografted rats with donor skin from the backs of other Wistar rats were takes at 30 days. Since the best results with Wistar autografts using dorsal skin were only 45 per cent takes, this 45 per cent might well represent the anticipated 100 per cent for this group, and thus the finding of 15 per cent takes in actuality represents a 33 per cent success in homografting. Of the 12 takes at 30 days, 2 were sacrificed at 34 days, 1 at 44 days, 8 at 60 days, and 1 at 78 days, at which times they all were still in good condition.

b. With Wistar-to-Wistar homografts similar to *a*, except that abdominal skin was used rather than back skin, there were 16 of 53 (30 per cent) takes.

Since Wistar autografts using abdominal skin were 97 per cent takes, this figure (30 per cent) need not be corrected. Its similarity to that obtained in *a* would indicate that, based upon the findings in 131 rats, a 30 to 33 per cent take is to be expected at 30 days after grafting, when Wistar animals serve as both donor and recipient for homografts. All takes in this group were observed for as long as 60 days, at which time they were sacrificed. Three had rejected by 40 days.

c. Where Long-Evans rats served as donors of abdominal skin and Wistar animals were recipients, at the end of 30 days only 8 of 202 grafted animals were found to be successful. Of the 8, 2 were sacrificed at 30 days, 4 at 40 days, and 1 at 70 days, all as takes, and one was rejected by 40 days.

Long-Evans recipients:

d. Homografts of abdominal skin in which Long-Evans animals served as both donor and recipient were practically 100 per cent nontakes by 30 days. Only one of 27 survived, and this animal rejected its graft by 45 days.

e. When Long-Evans rats served as recipients for Wistar abdominal skin, 14 of 193 (7 per cent) were still takes at 30 days. Thirteen of the 14 were of the +1 or +2 variety. Three became nontakes during a period of subsequent observation (by 70 days). The rest remained excellent until either sacrifice or death or are still alive. Five were sacrificed between the thirty-sixth and forty-second days, one died at 4 months, 4 are alive at 3 months, and one is alive at 5 months.

For the most part, homograft rejections in all of the preceding control groups could be ascertained as such by the tenth to fourteenth days, when ulceration, heavy crusting, and exudation were apparent. Occasionally, particularly when back skin was used, it was not until about the twenty-first day that a reject was definite.

3. Homografts in Vitamin B_6 Deficient Animals.—All animals, both donor and recipient, were made deficient prior to grafting by receiving a diet lacking in this vitamin for 3 to 4 weeks. Table III is a summary of the results for this group 30 days after grafting.

TABLE III. FATE OF HOMOGRAFTS 30 DAYS AFTER GRAFTING IN 188 WISTAR AND LONG-EVANS RATS DIETARY DEFICIENT IN VITAMIN B_6

DONOR	RECIPIENT	TYPE OF DONOR SKIN	NUMBER OF RATS	TAKES				NON-TAKES	PER CENT TAKES
				+2	+1	±	TOTAL NUMBER		
a. Wistar	Wistar	Back	62	21	21	14	56	6	90
b. Long-Evans	Wistar	Abdominal	73	5	9	0	14	59	19
c. Wistar	Long-Evans	Abdominal	53	26	7	0	33	20	62

Wistar recipient:

a. Homografts within the same strain, that is, Wistar-to-Wistar, using back skin, resulted in 90 per cent takes at 30 days. In this group, 27 of the 56 (48 per cent) takes resumed a normal diet on an average of 37 days (most between the third and fourth week) after grafting. Of these, 3 died (66, 76, and 77

days, respectively), 20 were sacrificed at intervals of time between 3 and 6 months, and 4, after longer than 8 months (Fig. 1, *A*), are still alive. The 29 not returned to a normal diet were maintained on the deficient diet until they eventually died of the deficiency. This occurred between 30 and 76 days. In no instance, whether or not animals were returned to a normal diet, was there found a breakdown of the graft during the period of observation after 30 days.

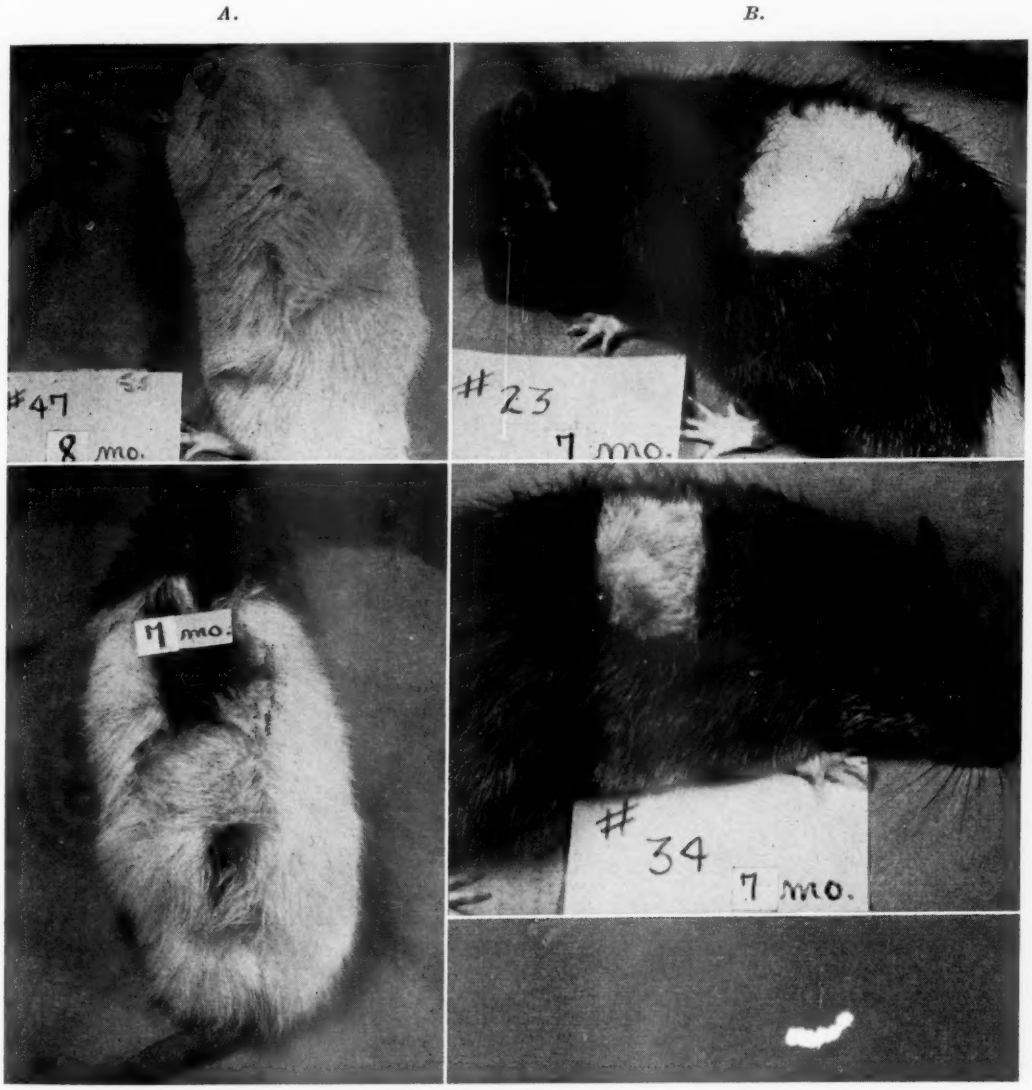

Fig. 1.—Representative prolonged homograft survivals. *A*, Wistar donor and Wistar recipient, both depleted of pyridoxine prior to grafting by vitamin B_6 deficient diet. *B*, Wistar donor and Long-Evans recipient, depleted by pyridoxine prior to grafting by vitamin B_6-deficient diet. *C* and *D*, Wistar donor and Long-Evans recipient treated with desoxypyridoxine subsequent to grafting.

b. When deficient Long-Evans rats served as donors of abdominal skin and deficient Wistars as recipients only 14 of 73 grafts were considered as takes at 30 days. Following return to a normal diet, 5 became nontakes at

42, 60, 60, 64, and 90 days, respectively, after grafting; 2 were sacrificed at 36 days; one died at 34 days; and 6, between 50 and 60 days, are still being observed.

c. With deficient Wistar rats as donors of abdominal skin and deficient Long-Evans rats as recipients, 62 per cent of 53 rats were successful at 30 days. Of the 33 takes, 8 were sacrificed between 30 and 36 days after grafting; one died at 31 days; one began to reject at 2 months, 4 at 3 months, and one at 5 months; 8 remain excellent takes after 6 months (Fig. 1, *B*); and 10 are excellent at 50 days and are being followed.

Since donor animals were in the same dietary state as recipients, the role of the deficient donor skin per se required study. What part did it play in the success of homografts in vitamin B_6 dietary deficient animals? To attempt to answer this question, skin from deficient Wistar donors was applied to normal Wistar recipients. The results, shown in Table IV, would indicate approximately the same percentage of successful grafts at 30 days as if normal donor skin had been used. Further, when normal donor skin was applied to deficient animals, the degree of success (66 per cent) approached that expected (90 per cent) had deficient donor skin been used. The slight difference that did occur is attributed to other than immunologic factors. The thinness of the deficient donor skin, in comparison to the thick normal dorsal skin, would seem to make the difference. It will be recalled that autografts in normal animals using dorsal skin were only 45 per cent successful.

TABLE IV. EFFECT ON HOMOGRAFTS (ALL BACK SKIN) USING DONOR SKIN FROM WISTAR RATS IN A VITAMIN B_6 STATE DIFFERENT FROM THE RECIPIENT (WISTAR) 30 DAYS AFTER GRAFTING

DIETARY STATE		NUMBER OF RATS	TAKES				NON-TAKES	PER CENT TAKES
DONOR	RECIPIENT		+2	+1	±	TOTAL NUMBER		
Deficient	Normal	67	2	11	6	19	48	28
Normal	Deficient	68	5	23	17	45	22	66

4. Homografts in Rats Receiving Desoxypyridoxine (DB_6).—As previously noted, animals in this group received 0.5 mg. of DB_6/100 Gm. of body weight intraperitoneally each day for 10 days, at the same time being on a vitamin B_6-deficient diet which was maintained for 5 additional days after stopping the DB_6 and before starting animals back on a 10γ vitamin B_6 diet. Abdominal skin was used for grafts in all animals. Results are summarized in Table V.

TABLE V. FATE OF HOMOGRAFTS 30 DAYS AFTER GRAFTING IN 212 WISTAR AND LONG-EVANS RATS RECEIVING DESOXYPYRIDOXINE (DB_6)

DONOR	RECIPIENT	TYPE OF DONOR SKIN	NUMBER OF RATS	TAKES				NON-TAKES	PER CENT TAKES
				+2	+1	±	TOTAL NUMBER		
a. Wistar	Wistar	Abdominal	41	29	1	3	33	8	80
b. Long-Evans	Wistar	Abdominal	73	5	5	13	23	50	32
c. Long-Evans	Long-Evans	Abdominal	22	1	0	0	1	21	5
d. Wistar	Long-Evans	Abdominal	76	35	19	8	62	14	82

a. When skin from Wistar donors was applied to Wistar recipients, 33 of 41 animals were still takes at 30 days; 91 per cent were of the +1 or +2 variety; 16 were sacrificed; and 17 of the 33 were observed further. Of the 17, 11 showed no tendency to change by 60 days. Two excellent takes at 30 days were nontakes at this time, and 4 showed slight hair loss or beginning contracture of the graft.

b. When Long-Evans animals served as donors for Wistar rats, 23 of 73 were considered takes at 30 days, and the majority of these were of the ± variety. It was disappointing to find that after putting animals back on a normal diet 3 weeks after grafting, all animals rejected their grafts by the fifty-first day.

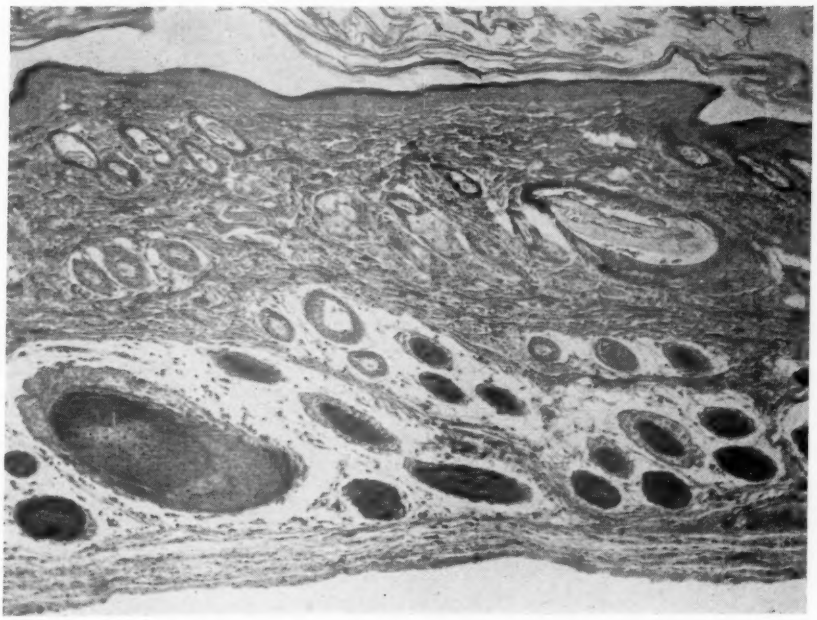

Fig. 2.—Homograft considered as +2 takes at 45 days. Skin appendages are preserved and dermal collagen appears normal (×75).

c. Contrary to expectations, Long-Evans-to-Long-Evans homografted animals treated with DB_6 did poorly. Only one of 22 (5 per cent), similar to control homografts, was successful. This animal was sacrificed after 35 days.

d. When Wistar animals served as donors of skin to be grafted on Long-Evans rats, which were subsequently treated with DB_6, results were remarkable. Of 76 animals grafted, 62 (82 per cent) were successful at 30 days following operation. A complete tabulation of the fate of these takes records that 14 became nontakes between 30 and 60 days, 10 more between 60 and 90 days, 7 between 90 and 120 days, and 2 at 5 months. Still under surveillance are 21 takes at 3 months and 5 takes over 6 months (Fig. 1, *C* and *D*). One animal died at 35 days and 2 were sacrificed for pathologic study at this time. Thus, 35 of 59 possible permanent survivors (60 per cent) were or are still good takes 3 or more months after grafting.

The variability of macroscopic features observed in homografts was also reflected in their morphologic appearance. Accordingly, 3 general groups could be recognized.

1. Those homografts corresponding to the +2 or +1 takes were characterized by an intact epidermis, abundant hair follicles with adnexa and dermal collagen which exhibited similar morphologic and tinctorial properties to those noted in successful autografts of normal skin (Fig. 2). The undulated, interrupted appearance of the collagen of successful homografts represented a distinguishing feature of grafts categorized in this group (Fig. 3). In addition, appropriate stains revealed elastic and reticulum fibers similar in appearance and distribution to those observed in normal skin. Inflammatory infiltrate appeared minimal, usually being restricted to the deep portion of the graft, and was comprised principally of lymphocytes with varying numbers of neutrophils and plasma cells. The vessels appeared mildly thickened but were otherwise not unusual.

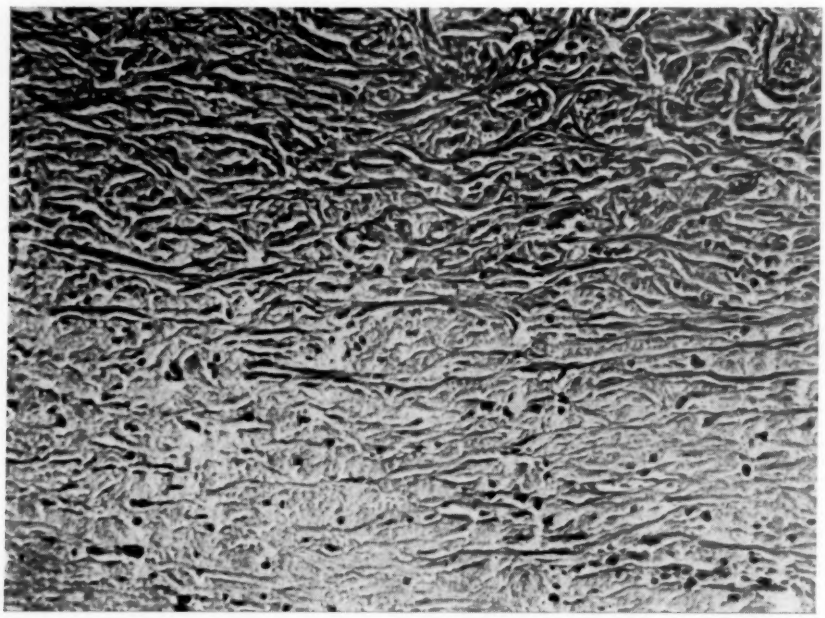

Fig. 3.—Difference in appearance of dermal collagen in portions of homograft. That portion which is wavy is considered as take whereas that which is homogenized is considered as nontake (×225).

2. Those grafts in which focal areas of hair growth were evident macroscopically and were classified as ± revealed islands of normal-appearing skin, as described previously, separated by relatively acellular, lightly staining, homogeneous collagen, which lacked the interrupted, undulated appearance of the former. The epidermis over these areas appeared atrophic and skin appendages were sparse, appearing trapped within this dermal scar. Relatively few discontinuous elastic fibers could be identified in such areas. This desmoplastic reaction appeared to arise principally at the sites of anastomosis

and base of the graft and to replace the corium of the graft by a process of "creeping substitution" (Fig. 4). Inflammatory components and vascular alteration were similar to those observed in successful homografts described previously.

Fig. 4.—Low-power appearance of homograft at 80 days considered as ± take. The normal-appearing skin is being replaced by scar from the base (×75).

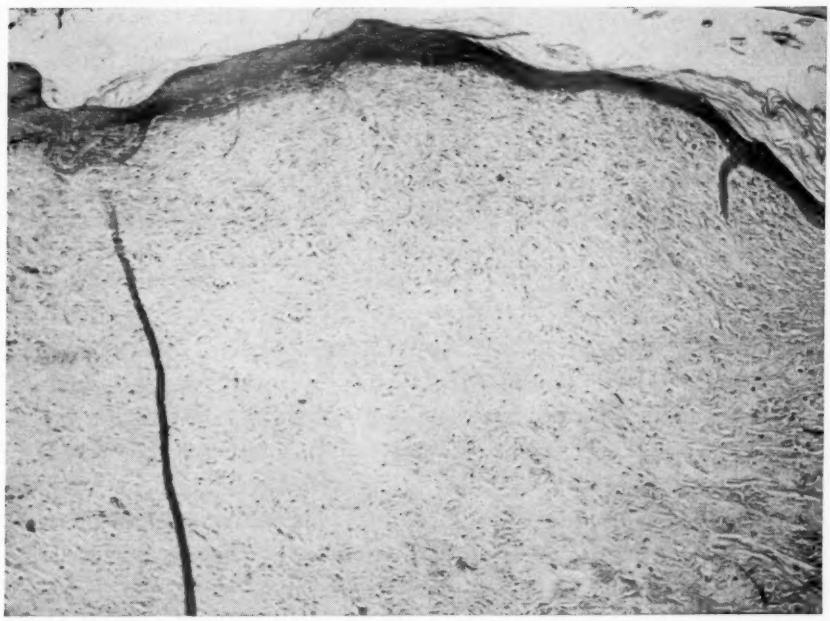

Fig. 5.—Appearance of homograft considered as nontake at 35 days. The dermal collagen is homogeneous and without appendages (×75).

3. Those grafts which were considered to represent nontakes and grossly appeared as scars morphologically appeared as an advanced stage of the desmoplastic reaction noted in those examples considered as ± (Fig. 5). Normal-appearing dermal collagen was not evident, and skin appendages were sparse. Although some of the latter appeared intact, many were observed in various stages of degeneration not infrequently accompanied by a foreign body reaction. It is to be noted that the intense inflammatory reaction and necrosis observed early in the process of homograft rejection were usually not evident in the grafts at the time of their removal for histologic study (after 30 days). However, such a process was observed in several homografts which appeared macroscopically to represent +2 takes but which at 60 or more days exhibited loss of hair (Fig. 6). Vasculitis was evident in such grafts in addition to infiltration with neutrophils, lymphocytes, and plasma cells, and degeneration of dermal appendages.

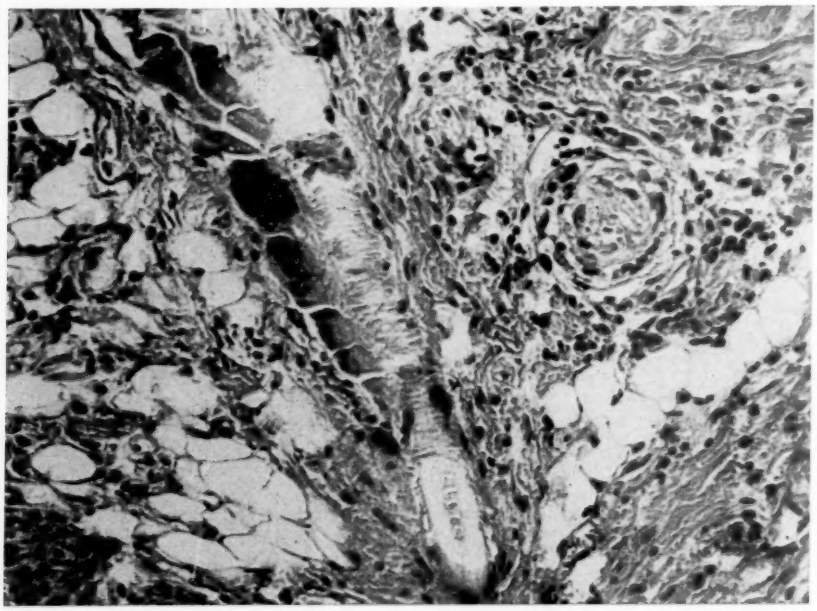

Fig. 6.—Vasculitis and degeneration of hair follicle with foreign body reaction in homograft at 111 days (×225).

DISCUSSION

A wealth of evidence would seem to implicate an antigen-antibody interaction as the cause of homograft rejections. The data presented in this paper would seem to strengthen this concept, for it has been adequately demonstrated that pyridoxine, and other vitamins as well, is important in the metabolism of proteins,[32] amino acids,[47] and, more specifically, antibody formation.[2] A source of antigen in the form of skin homografts has been presented to rats previously made deficient in vitamin B_6, and the host response, though variable, was different from that of nondeficient animals. It might seem reasonable to attribute the initial survival of grafts to an inability of the

host to produce antibodies because of the pyridoxine-deficient state. The actual fashion in which pyridoxine deficiency disturbs antibody synthesis is not fully understood. Similarly, DB_6 has been shown to depress not only primary antibody production to antigens but also the anamnestic rise to a reinjection of antigen.[1, 51] That its effect is by the production of a vitamin-deficient state has been demonstrated by the injection of DB_6 to animals fed a control[10] vitamin B_6 diet plus parenteral pyridoxine (1 mg. daily). No successful survivals of grafts were obtained.

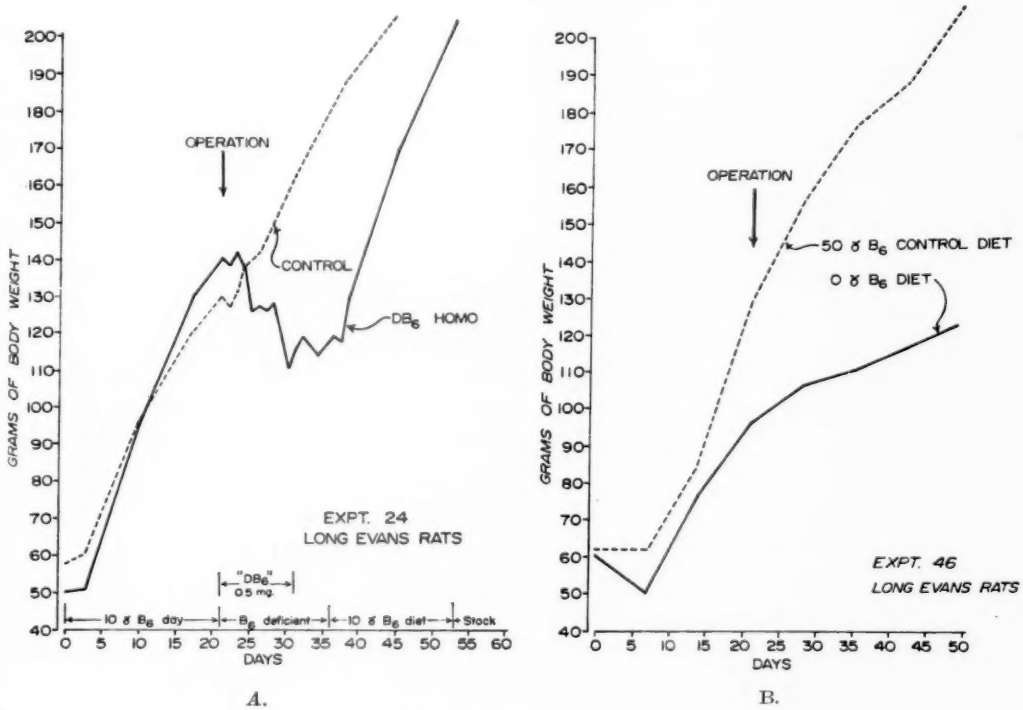

Fig. 7.—*A*, Typical growth curve in desoxypyridoxine (DB_6) experiment. *B*, Typical growth curve in dietary deficient vitamin B_6 experiment.

The effects of either a deficient state of vitamin B_6 produced dietarily or by the administration of DB_6 are similar and dependent upon the degree of avitaminosis. In either, weight loss occurs in a fashion typically demonstrated in Fig. 7. Acrodynia (that is, a scaly condition of the skin, ears, paws, and snout), edema of the corium, contracture and dermatitis of the tail, anemia, lymphopenia, and atrophy of lymphoid elements, particularly the thymus, have all been described. Depression of antibody synthesis may occur before the appearance of such severe clinical manifestations. In the present experiments it was not possible to correlate weight loss or other signs of deficiency with successful survivals of grafts. In fact, many Wistar animals showed extreme effects, and grafts were not tolerated. Many Long-Evans animals displayed little or no evidence of deficiency but had +2 takes of homografts.

Because of weight loss in the deficient animals, it was deemed essential to estimate the effect of inanition upon graft survival. Such previous studies

with diphtheria toxoid and human red cells as antigens have indicated that this was not a factor. Antibody response was not modified by inanition.[2] Similarly, skin homografted inanition control animals suffered a destruction of their grafts at a time equivalent to that in normal homografted rats.

Consideration has been given to the possibility that perhaps the antigenicity of the donor skin has become modified by the deficient state of the donor animal and that this might account for at least partial success of grafts. Based upon evidence presented, this does not seem likely. However, that the grafted skin, either from a normal or deficient donor, is not "altered" after application to an already deficient recipient or one receiving DB_6 has not been disproved.

Although a reasonable explanation for the initial survival of grafts might be presented, it becomes more difficult to account for the protracted and, in some instances, seemingly permanent takes after a return to normal vitamin B_6 intake. It would seem that a form of acquired tolerance has been achieved, at least to an initial skin homograft, for, as has been commented upon by Billingham and associates,[8] "a tolerated homograft does not merely 'contain' antigens; it continues to be a source of antigenic stimuli—or rather, of stimuli which would be antigenic to a host with normal powers of response." It has been demonstrated that when animals with vitamin B_6 deficiency and depression of antibody synthesis are returned to a normal diet or given parenteral vitamin B_6 there is a gradual restoration of the capacity to produce antibodies. With the administration of human red cells as the antigen to animals depleted of pantothenic acid or pyridoxine and then given large doses of these vitamins, antibody titers become pronounced only after 3 to 4 weeks.[39] In such rats, similar to what was observed in these experiments, there was an immediate growth response. Therefore, with return to a normal diet, after several weeks, rejection of the graft might be anticipated. This was the case in numerous instances. However, this did not occur universally, and several possibilities suggest themselves as to why grafts have survived 4 to 6 months or longer. Perhaps in such instances permanent damage to the immunologic system of the animal has occurred which was not reversed after vitamin repletion. Or perhaps the vitamin-deficient state of the animal results in a regression of the immunologic system to a "state of immaturity" and the antigen of the graft leaves its mark, similar to that in the actively acquired tolerance of Billingham and co-workers,[8] so that with restoration of the normal state a partial or total acceptance of the graft ensues. Of interest in this regard will be the fate of "second-set" grafts from the same donor that contributed the primary piece of skin.

Might not the mechanism of survival be based upon the so-called "critical period," as suggested by Woodruff?[56] It has been suggested by him that homotransplants become progressively less vulnerable as time goes on and, after a certain period, are capable of surviving in face of the development of a high degree of immunity in the recipient. The supposition in this study would be that grafts were protected during this "critical period" by temporary suppression of the immune response by vitamin B_6 deficiency. As Eichwald[26]

has commented, this would seem to imply that a change of antigenicity and the immunologic characteristic of the graft occurs, or that it is replaced by host tissue.

It is fair to state that the mechanisms and the specificity of these findings are only speculative.

No obvious explanation at present can be offered for the finding that Wistar donor skin applied to vitamin-deficient Long-Evans animals resulted in better survival rates than when Long-Evans donor skin was grafted to deficient Wistar rats. Information as to the reason for the difference in this response might well be fundamental to a better understanding of the mechanism of homograft rejection. It is to be emphasized that in dietary vitamin B_6-deficient or DB_6 injected Wistar rats homografts with skin from Long-Evans animals fared better than did those in the control group. By 10 to 14 days almost 100 per cent of control animals had completely rejected their grafts, whereas at this time in deficient animals the number of takes were great; rejection did not occur until after 21 to 30 days.

The 30 per cent survival of Wistar-to-Wistar control homografts would suggest that there is a considerable degree of genetic similarity within this strain in contrast to the Long-Evans rats which demonstrated approximately a 5 per cent successful take of normal homografts. The possibility, although slight, that a few of the successes in either strain might be due to the fact that numerous control animals were on a 10γ vitamin B_6 diet for 3 to 4 weeks prior to grafting has been considered. Perhaps this amount of the vitamin was inadequate and a deficiency state (in relation to antibody production) was in effect. The finding of almost 100 per cent takes in homografts exchanged between dietary vitamin B_6-deficient Wistar animals and of 80 per cent when these rats received DB_6 would suggest that the genetic barrier was easily overcome. In contrast, when Long-Evans rats were homografted within their own strain, results were poor despite a vitamin B_6 deficiency.

The marked superiority of abdominal skin over back skin in autografts would suggest that the former be used exclusively in skin grafting of rats. Its use eliminates the necessity for split-thickness grafts. The thinness of the abdominal skin would appear to be its chief asset, for back skin in deficient animals, which is thinner than normal, results in almost as many autograft survivors as does normal donor skin from the abdomen.

Complete significance of the findings presented remains to be elucidated. Mechanism, specificity, the effect of other vitamin deficiencies, specific dosage of antimetabolite (DB_6) required, the fate of "second-set" grafts, and clinical application are all being investigated. It would be hoped that the use of DB_6 in the human would result in an immunologic response to grafting similar to that observed in the Long-Evans rat. In any event, a method heretofore undescribed has been presented which has resulted in prolongation and, in many instances, seemingly permanent survival of skin homografts in the rat.

SUMMARY AND CONCLUSIONS

It is generally believed that homograft failure is due to an immunologic response of the recipient to the graft. Ample experimental evidence has

demonstrated the importance of vitamin B_6 in antibody synthesis. Deficiency of this vitamin, produced by diet or by the administration of desoxypyridoxine (DB_6), the specific pyridoxine antagonist, results in an impairment of antibody response to antigenic stimulation. Because of this it was deemed worthwhile to evaluate the effect of pyridoxine deficiency upon the fate of skin homografts in Wistar and Long-Evans rats. Results of autografts, control homografts, homografts in animals made deficient in vitamin B_6 by diet prior to grafting, and in animals administered desoxypyridoxine subsequent to grafting are reported. In this study 1,529 rats were utilized. Findings of note are as follows:

1. Thinner abdominal skin is superior to back skin for grafting in the rat.

2. Greater genetic similarity exists in Wistar rats than in Long-Evans rats. Control homografts between nonlittermated members of the Wistar strain resulted in a 30 per cent survival, while only 4 per cent of the Long-Evans animals under similar conditions were takes 30 days after grafting.

3. Homografts of skin from control-fed Long-Evans donors to control-fed Wistar recipients were 4 per cent successful at 30 days after grafting; similar grafts from Wistar donors to Long-Evans recipients demonstrated a 7 per cent survival.

4. Nonlittermated Wistar rats, serving as both donor and recipient, when fed a diet deficient in vitamin B_6 prior to grafting demonstrated a 90 per cent survival of homografts after 30 days. Similarly, 62 per cent were takes when Long-Evans rats served as recipients for Wistar-donated skin. In both groups, after return to a normal diet, survivals of grafts have been observed for longer than 6 months. Less impressive results were obtained when pyridoxine-deficient Wistar animals served as recipients for skin from depleted Long-Evans rats. However, even these fared better than their vitamin B_6-fed controls.

5. Animals injected with desoxypyridoxine subsequent to homografting demonstrated a difference in response from control rats not so treated. Homografts of Wistar rats (both donor and recipient) receiving desoxypyridoxine were 80 per cent successful after 30 days. As with animals depleted of vitamin B_6 by diet, Wistar recipients did not tolerate grafts of Long-Evans skin as well. Long-Evans rats grafted with Wistar skin and subsequently receiving desoxypyridoxine demonstrated an 82 per cent survival of grafts 30 days after grafting, and a large number of these have remained excellent for more than 6 months.

6. A discussion of possible mechanisms responsible for the initial and protracted survival of homografts has been presented.

It is concluded that significant prolongation and, in many instances, apparent permanent survival of skin homografts in rats has been accomplished by a method heretofore undescribed. Results would favor further study of this approach to the modification of the host response to homografting in the hope that clinical application would be practical.

Acknowledgment is made of the devoted assistance of Mr. Don Gwillim and Miss Thelma Moroso in this project.

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DISCUSSION

DR. JOSEPH E. MURRAY (Boston, Mass.).—To my knowledge, there are no pure genetic strains of rats and, as Dr. Fisher pointed out, a certain number of homografts survived without any form of treatment or dietary deficiency. If we study the homograft problem, especially with skin, it would seem advisable to use either mice, of which there

are pure strains, or go to a homologous system completely, like dogs or rabbits, so that we do not have the problem of a certain base line of success of homotransplants between littermates or between animal colonies. At the recent meeting in transplantation, pure strains of guinea pigs that can be obtained were described. There are also pure strains of fish.

What was the general health of these rats? As we know from clinical work in homografting uremic patients or homografting burn patients, the homografts survived for a long time and sometimes they seemed almost permanent. That is attributed to the general decline in health of the person or the host. Have you had any correlation, even in the group that you studied, between the general state of health and the acceptance?

DR. FISHER (Closing).—The general health of the rats and the other points, too, are included in the text of the paper. There was no correlation between the state of deficiency in these animals with the graft survival. Some animals that exhibited clinical signs of deficiency rejected grafts and, in others which did not exhibit signs of deficiency, the grafts survived.

Insofar as the use of the rat is concerned, this is why we used 1,500 rats. We wanted to have statistically significant numbers. We feel that we have adequately controlled our homograft series, and, although we find that there is a 30 per cent homograft survival when 2 Wistar rats are used, this was not the case when Long-Evans rats were used, nor was it the case when Wistar rat skin was put on Long-Evans animals.

But we do appreciate the fact that the rat is what you said it is and we are doing these experiments in dogs and other animals, too. We did not use littermated animals. In fact, animals that were homografted actually came from 2 different suppliers.

We did inanition controls because some of these animals lost weight, and grafts did not take in inanition control animals.

THE EFFECT OF L-NOREPINEPHRINE UPON THE MYOCARDIAL OXYGEN TENSION AND SURVIVAL IN ACUTE HEMORRHAGIC HYPOTENSION

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(From the Department of Surgery, Western Reserve University School of Medicine and City Hospital)

INTRODUCTION

In a previous publication,[5] data were presented which indicated that, during hypotension which resulted from arterial bleeding, l-norepinephrine restored the arterial blood pressure to normal and increased the rate at which blood flowed through the coronary vessels. Paradoxically, the data on survival from those same experiments suggested that such improvement in the myocardial circulation and in the systemic blood pressure had no significant effect upon the mortality from the results of hemorrhage. Further information was needed to explain this discrepancy.

The development of the platinum electrode for measuring oxygen tension in tissues[7] has provided a tool for examining the effectiveness of a primary function of the circulation, namely, that of maintaining an adequate supply of oxygen in tissues to permit normal cellular metabolism. Therefore, experiments were planned to test, by means of the platinum polarograph, the question of whether or not a sympathomimetic agent would increase the tension of oxygen in the myocardium during oligemic hypotension.

Furthermore, it was thought that, in the previous experiments in which the hypotension of oligemia was corrected by a sympathomimetic agent only after a lapse of at least 10 minutes, possibly even in this short period of time essential cells in vital structures may already have died. Thus it was decided to test the effect of l-norepinephrine upon the survival rate of animals which had been bled and made hypotensive for less than 5 minutes.

METHODS

A. Studies of Myocardial Oxygen Tension.—Experiments were performed in healthy adult mongrel dogs of both sexes weighing between 8 and 15 Kg. Sodium pentobarbital (27 mg. per kilogram of body weight) was injected intravenously for anesthesia. The blood pressure was measured from the proximal end of the transected right common carotid artery either by a Hürthle manometer recording on a smoked-drum kymograph or by means of a strain gauge and direct-writing oscillograph. The left common femoral vein was cannulated for intravenous injections. The heart was exposed through a left intercostal

Supported by Research Grant No. 800 of the Cleveland Area Heart Society and the Prentiss Fund.

Read at the Annual Meeting of the Society of University Surgeons, Boston, Mass., Feb. 13-15, 1958.

*Research Fellow of the Cleveland Area Heart Society.

incision. The pericardium was incised ventral to the phrenic nerve. This brought clearly into view the left ventricle with the branches of the left coronary artery, the left auricular appendage, and the pulmonary artery. It was a simple matter to separate the descending branch of the left coronary artery from its companion vein and to apply an occluding bulldog clamp on artery and vein separately or together.

The tension of oxygen in the myocardium was measured by means of the open-type platinum electrode, using a technique based upon that described by Davies and Brink.[7] Instead of using capillary glass tubing for insulation, a plastic coating was adopted.[10] Platinum wire (diameter 0.2 mm.) was smoothly dipped in Hysol* by means of an electric clock motor adapted for this purpose and then baked in an oven passing through the temperatures of 70° to 170° C. and return in one hour. This entire process was repeated 10 times. One end of the coated wire was then soldered to a fine, very flexible, insulated copper wire using a resin-core solder; the opposite end was exposed flush with the plastic coating. The electrode was then tested in aerated and in oxygen-poor saline solutions and, if satisfactory responses were obtained, was considered ready for use. The potential difference applied was usually 0.6 volt. Occasionally, 0.8 volt was used with no difference in the results.

Two to five of the electrodes were carefully imbedded in the myocardium at points along the distribution of the descending branch of the left coronary artery. Experiments were performed with the chest open and the animal on artificial respiration or with the chest closed and the animal breathing on his own. In the experiments in which the chest was closed, the electrodes were led out together through the incision which was sutured tightly around the wire leads from the electrodes. The electrodes were fastened into the myocardium by means of fine silk sutures through the epicardium to minimize possible movement and dislodgment of the electrodes. Lead II of the electrocardiogram was recorded. Values for oxygen tension were read from the galvanometer in microamperes and were recorded as percentage of the control galvanometer readings. The reference electrode was a chlorided silver electrode imbedded in the skin. A calomel half-cell was used in the circuit in some of the experiments.

After the necessary surgical procedures had been completed, the animals were heparinized with 5 mg. of heparin per kilogram of body weight. A control period of 20 minutes was allowed for preliminary observation. During this time, the values for blood pressure and oxygen tension were always stable. Rapid bleeding was effected by inserting a cannula into the left femoral artery and connecting it to a bottle held at such a level as to permit a drop of the right carotid arterial pressure to 30 to 40 mm. Hg in less than 5 minutes. The hypotension was maintained for 2 to 6 minutes. At the end of this time, l-norepinephrine in isotonic saline solution was infused at the rate of 22 μg per minute. A total volume of 30 to 40 ml. was injected. The blood pressure was thus maintained at prehemorrhagic levels (110 to 116 mm. Hg). This period of oligemic normotension was maintained for 30 minutes. At the end of this time, the infusion of l-norepinephrine was stopped. The animals again became hypotensive; the blood pressure fell to a mean level of 55 mm. Hg. After a total duration of 20 minutes for this degree of hypotension, the animal's own blood was reinfused.

B. Survival Experiments.—Experiments to test the effect of l-norepinephrine upon survival from oligemia were performed in 17 pairs of animals according to the procedure previously reported.[5] In the present experiments, however, the l-norepinephrine was administered as soon as possible after attaining the desired volume of bleeding into the reservoir bottle. This was always achieved in less than 5 minutes from the start of bleeding. During this time, the blood pressure usually fell to levels too low to read. However, on the basis of the paired controls, it is known that this extent of bleeding, approximately 4 to 5 per cent of the body weight (Table I), would have produced a blood pressure settling between 30 and

*Houghton Laboratories, Inc., Olean, N. Y.

40 mm. Hg within 5 to 10 minutes after the start of the bleeding. The oligemia was continued for 2 hours unless the animal died before this time. Autopsies were promptly performed on animals that died during the tests. The survivors were autopsied on the seventh day after the experiment.

These experiments were performed under strict aseptic conditions. No antibiotic was used. In none of the survivors was there any evidence of infection in the wounds which had been made for the necessary exposure of blood vessels to be used for bleeding, recording of the blood pressure, and infusion.

RESULTS

A. The Effect of Bleeding Upon the Tension of Oxygen in the Myocardium.—In every one of the 19 dogs in which the experiment was done, the tension of oxygen dropped as bleeding progressed (Fig. 1). This occurred in animals with closed or open chest and with intact or impaired coronary arterial circulation. The average drop in oxygen tension was 39 per cent of the control value; the minimum and maximum were 15 and 87 per cent, respectively. The electrocardiograms showed depression of the S-T segment and occasional inversion of the T wave.

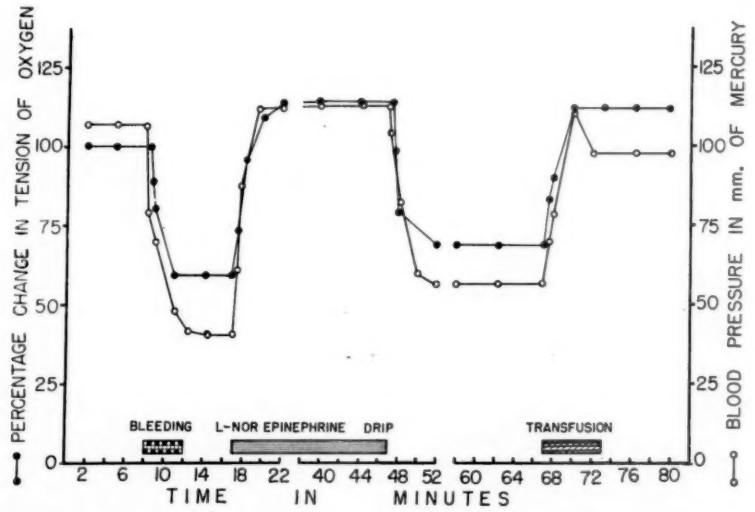

Fig. 1.—Changes in blood pressure (open circles) and in oxygen tension (dots), related to bleeding, infusion of l-norepinephrine, cessation of the infusion, and transfusion of blood from reservoir. Oxygen tension (scale on left) is expressed as percentage of the control value which is taken to be 100 per cent. Blood pressure (scale on right) is recorded in millimeters of mercury. All points represent means of 16 experiments.

B. The Effect Upon Myocardial Oxygen Tension of Restoring the Blood Pressure to Normal by Means of l-Norepinephrine Without Correcting the Oligemia.—In all cases, restoration of the blood pressure to the prebleeding level, or slightly above, by the infusion of l-norepinephrine was accompanied by an increase in myocardial oxygen tension, whether the chest was open or closed and whether the coronary arterial circulation was intact or impaired by clipping the descending branch of the left coronary artery near its angle of descent. In fact, the value for the oxygen tension as measured by this technique during the administration of l-norepinephrine averaged 13 per cent higher than the prebleeding level. This level was maintained for the duration

of the infusion (30 minutes). When the infusion of l-norepinephrine was discontinued, both the blood pressure and the oxygen tension dropped but not quite as low as before (Fig. 1).

In 3 experiments, the rise in the tension of oxygen following the intravenous infusion of l-norepinephrine was preceded by a very brief (30 seconds) sharp dip in the oxygen tension (Fig. 2). This implied that the increased rate of coronary arterial blood flow may have been preceded by a vasoconstriction[1] in those 3 animals.

Transfusion of the blood collected in the reservoir into the same animal restored the blood pressure to nearly normal and the myocardial oxygen tension to slightly greater (10 per cent) than control values. None of the animals in this group died during the experiment. Cardiac irregularities were encountered in every animal as frequent premature systoles.

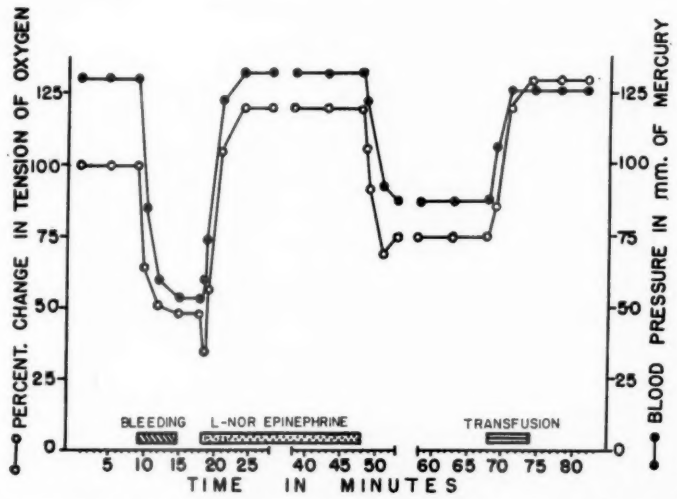

Fig. 2.—Data presented as in Fig. 1 except that blood pressure is represented by dots and oxygen tension by open circles. All points represent means of 3 almost identical experiments. Note the dip in oxygen tension from the hypotensive low level before the rise to above normal levels induced by the infusion of l-norepinephrine.

TABLE I. THE EFFECT OF L-NOREPINEPHRINE UPON SURVIVAL FROM ACUTE HEMORRHAGIC OLIGEMIA

GROUP	TOTAL NUMBER OF ANIMALS	BODY WEIGHT (KILOGRAMS)	AMOUNT BLED (PER CENT OF BODY WEIGHT)	RANGE OF MEAN ARTERIAL PRESSURE DURING HYPOTENSION (MM. HG)	DURATION OF HYPOTENSION (MINUTES)	SURVIVORS	
						NUMBER	PER CENT
l-Norepinephrine	17	10 ± 2.8	4.3 ± .69 S.E. = 0.16	5 to 25	< 5	15	88*
Paired control	17	10 ± 2.4	4.5 ± 0.6 S.E. = 0.14	30 to 40	50 to 120	6	35*

*P < .01.

C. l-Norepinephrine and Survival From Acute Hemorrhagic Oligemia.—Of the 17 animals whose hypotension, but not the oligemia, was corrected by the administration of l-norepinephrine, all but 2 survived, a survival rate of 88 per cent (Table I). The 2 which died did so 60 and 100 minutes, respectively, after bleeding had been started. In one of them, the blood pressure

failed to stabilize after having been drained of blood amounting to 5 per cent of the body weight. The animal had become apneic during the bleeding and for 10 minutes afterward, during which time artificial respiration had to be maintained. l-norepinephrine had no demonstrable effect upon the blood pressure in this animal. It died 100 minutes after bleeding. It had not developed any gross cardiac irregularity. The second animal developed ventricular fibrillation 60 minutes after bleeding and was not resuscitated.

The 2 animals which died received l-norepinephrine at the rate of 150 μg of the base per minute. The animals which survived were given approximately 40 μg per minute. The amount used in any case was determined not arbitrarily, but by the amount needed for restoring and maintaining the blood pressure at the prebleeding level.

Of the 17 paired control animals which were allowed to remain hypotensive and oligemic, only 6 survived, a survival rate of 35 per cent. All those that died did so during the experiment, with 4 exceptions. These 4 died within 12 hours later. They had never recovered consciousness. The 7 animals which did not survive the experiment died between 50 and 110 minutes after the beginning of the bleeding. In this group of 7, the heart was found to be in standstill at immediate thoracotomy. None showed fibrillation.

DISCUSSION

The data from these experiments indicate that a decrease in myocardial oxygen tension occurs when the systemic blood pressure drops as the result of hemorrhage (Fig. 1). Since this fall in oxygen tension occurs despite the fact that the work of the heart during this period is actually decreased, it is reasonable to attribute the finding to impaired transport of oxygen to the myocardium by the coronary circulation. Measurements of the rate of coronary blood flow by indirect methods during hypotension resulting from hemorrhage have yielded conflicting results, despite the fact that all are agreed that the myocardial vascular resistance is decreased during the oligemic hypotension. This decreased vascular resistance cannot be expected to counteract the reduced head of pressure in the main coronary arteries which occurs when the systemic blood pressure falls to very low levels. However, since these 2 influences, resistance and pressure, operate in opposite directions, it is possible to have various summations of effects depending upon the degree to which pressures are reduced. This may account for the fact that some investigators[8] have reported decreases in the rate of coronary arterial blood flow during hemorrhagic hypotension, while others have reported only insignificant changes.[5]

The data further demonstrate that the increased rate of blood flow in the myocardium reported in previous experiments in which l-norepinephrine was administered during oligemic hypotension does in fact restore the oxygen tension of the myocardium to normal or above (Fig. 1). One might argue that this return of oxygen tension to normal could represent a failure of oxygen utilization by the myocardium. Burdette[3, 4] and Wilhelmi[4] showed that, in

the rat, alterations do occur in the rate at which myocardial slices utilize oxygen at different times after hemorrhage. Depressions in oxygen consumption, however, occurred late and not early. Furthermore, in our experiments, the rise in myocardial oxygen tension was temporally very closely related to the administration of the sympathomimetic agent and the consequent rise in blood pressure. There is no evidence that sympathomimetic agents depress the rate of oxygen consumption in the myocardium. Finally, other experiments have shown that with the administration of l-norepinephrine the rate of oxygen consumption by the heart is increased and the coefficient of extraction from the coronary blood is decreased despite the evidence that the work of the heart is actually increased.[5] It would seem fair to conclude, therefore, that the administration of l-norepinephrine during hemorrhagic hypotension can improve the circulation of the heart and thereby restore to normal the tension of oxygen in the myocardium and the availability of oxygen to cells that need it.

Despite this and other evidence that the myocardial ischemia which occurs during oligemic hypotension is corrected by the administration of l-norepinephrine, there have been up to this time no objective data to demonstrate that the survival of animals subjected to severe hypotension from bleeding is improved by the use of sympathomimetic agents. In fact, most recently, Close and associates[6] have shown that the mortality from acute hemorrhagic hypotension is doubled when l-norepinephrine is used. The survival data reported here are therefore of particular interest.

The difference between these and previously reported survival experiments is that the hypotension in the present experiments was not allowed to persist for more than 5 minutes. In fact, in most instances, the duration of the hypotension was considerably less than 5 minutes. There would seem to be a critical duration of ischemia, then, beyond which vital cells and, eventually, the entire animal die. This critical interval under the conditions of these experiments is about 5 minutes. The evidence is that oligemic hypotension of a given degree and duration will prove lethal to a certain proportion of animals, 65 per cent in the present series. A sympathomimetic agent which under such circumstances will improve the circulation of the myocardium and, probably, of the brain as well[2] can prevent the development of lethal changes in that proportion of animals. For those in the experimental population whose vital organs are less sensitive to the effects of hypotension or ischemia of tissues, the administration of such agents may not have a beneficial effect or, as in the experiments of Close and co-workers,[6] may actually be injurious.

It is noteworthy that, in our present survival experiments, the animals that died lived no more than 12 hours after the experiment during which time none had regained consciousness. It may be argued that the most likely organs whose failure would lead to such rapid death are the heart and brain. One would expect that lethal changes in the kidneys, liver, gastrointestinal tract, endocrine glands, and other organs would permit longer survival. However, in man, the effects on these other organs may well be of greater importance than they appear to be in the dog.

Obviously, correction of the oligemia is the most effective over-all treatment of hemorrhagic hypotension. This is both curative and preventative when the hypotension has been of such short duration that lethal changes have not taken place in vital cells. Beyond this time, restoration of blood volume may not permit survival of subjects who have developed lethal changes in vital structures, but at least it will not kill those who, by virtue of the metabolic properties of their vital cells, would have survived an unavoidable period of hypotension. As temporary expedients, sympathomimetic agents may be helpful if hypotension has not existed beyond a very few minutes. If the hypotension has been of longer duration, these agents are ineffective and can themselves cause death by their untoward effects.[9]

SUMMARY

The tension of oxygen in the myocardium was recorded by means of the platinum polarograph in 19 heparinized dogs with sodium pentobarbital anesthesia under the following conditions after a control period of 20 minutes: (1) rapid bleeding with systemic arterial blood pressures at 30 to 40 mm. Hg, (2) restoration of the blood pressure to control levels without correcting the oligemia, (3) return to the hypotensive state, and, finally, (4) correction of the oligemia and hypotension by transfusion of the reservoir blood. The tension of oxygen in the myocardium fell sharply with the fall in blood pressure, rose promptly with the rise of blood pressure when l-norepinephrine was injected intravenously, fell again when the l-norepinephrine was discontinued, and returned nearly to the control levels when the reservoir blood was transfused (Fig. 1). In 3 animals, the rise in myocardial oxygen tension when l-norepinephrine was injected was preceded by a very brief drop (Fig. 2).

Survival experiments were performed in 17 pairs of dogs. Each pair was subjected to rapid bleeding at the same time. One animal served as control; the other received a constant injection of l-norepinephrine starting 5 minutes or less after the beginning of bleeding and severe hypotension. Animals which survived 2 hours from the beginning of the bleeding were transfused with their own blood and returned to their cages. The survivors were autopsied at the end of one week. Of the 17 animals treated with l-norepinephrine, 2 died before the end of the 2 hours of the experiment (Table I). Those animals that survived the experiment survived until autopsy a week later. Among the 17 paired control animals, 11 died (Table I). Seven died during the 2 hours of the experiment; 4 died within the next 12 hours.

l-Norepinephrine corrects the myocardial ischemia which occurs during severe hypotension from bleeding and increases the chances of survival provided the hormone is administered less than 5 minutes from the beginning of severe hypotension under the conditions of the experiments reported here. Other evidence indicates that, if administered 10 minutes or longer after the onset of severe hypotension, l-norepinephrine does not have a beneficial effect and may be injurious.

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Discussion

DR. JAMES D. HARDY (Jackson, Miss.).—Dr. Simeone, do you use Levophed now when all looks hopeless?

DR. SIMEONE (Closing).—That question is not as easy to answer as it would seem. We do use Levophed when everything else seems hopeless, but we use it in a hopeless manner. The difficulty is that, as the data show, you can in fact increase the rate of blood flow through the myocardium and, as the work of Dr. Bloor has shown, through the brain as well. But, much as in the problem of cardiac arrest, lethal changes occur very quickly in vital areas and, if they are not corrected within a very brief period of time, the use of these agents may do more harm than good because of the many side effects that they produce.

INDUCED LYMPHOSARCOMA RESULTING FROM THE TRANSPLANTATION OF SKIN GRAFTS FROM ANIMALS TREATED WITH 20-METHYLCHOLANTHRENE

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(From the Departments of Surgery and Microbiology, University of Kansas Medical Center)

ORIGINALLY this study was started with the purpose of inducing tumors in mice with methylcholanthrene. Six purebred strains of mice were selected for painting with methylcholanthrene. Our initial thesis was an attempt to alter the factors responsible for the acceptance of skin grafts from a donor animal treated with a carcinogen when transplanted to a dissimilar strain of mice.

METHODS

A 0.6 per cent solution of 20-methylcholanthrene in a reagent grade of benzene was applied to the dorsal unepilated interscapular skin of mice with a No. 4 camel's-hair brush. A single stroke of the brush delivered about 0.1 mg. of methylcholanthrene.[6] Painting was done daily for nine weeks. The following pure strains of mice were selected for painting: Balb/c, C57B1/6, A/Jax, C57Br/cd, DBA/1, and DBA/2. Squamous cell tumors were induced in all of the above strains by skin painting. Only 4 of the DBA/1 mice survived. Four months later, after a squamous cell carcinoma appeared in the painted dorsal skin area of these DBA/1 mice (Fig. 1), full-thickness skin grafts 1 sq. cm. in diameter were taken from the ventral surface. These grossly normal-appearing skin grafts were transplanted to patterned dorsal surgical defects of 10 untreated normal DBA/1 mice. As a control 10 CFW mice also received the same skin transplants. Fixation of the grafts was done with interrupted sutures of 5-0 black silk. A portion of the induced squamous cell carcinoma was transplanted subcutaneously to the flank of 10 normal DBA/1 and 10 normal CFW mice. A No. 15 trocar was used to transplant the cancer tissue.

RESULTS

Epilation was noted on the sixth day in the painted areas with scaling of the epidermis. Papillomas appeared on about the thirty-sixth day. Squamous cell carcinomas appeared at the end of 9 weeks.

The induced squamous cell carcinomas transplanted subcutaneously to 10 normal DBA/1 mice survived and had the same microscopic characteristics of the original tumor. None of these animals died from these transplanted tumors. None of the squamous cell carcinoma transplants survived in 10 CFW mice.

Isologous skin grafts taken from the ventral surface of painted DBA/1 mice were accepted and grew in the 10 DBA/1 normal mice. The gross appearance

Supported in part by the American Cancer Society and Fellowship of the American Tobacco Company.

Read at the Annual Meeting of the Society of University Surgeons, Boston, Mass., Feb. 13-15, 1958.

*Fellow of the American Tobacco Company.

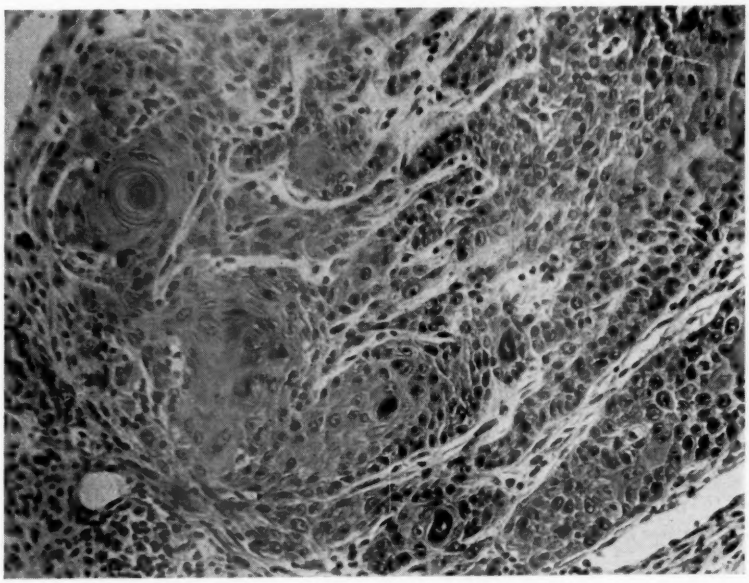

Fig. 1.—Induced squamous cell carcinoma.

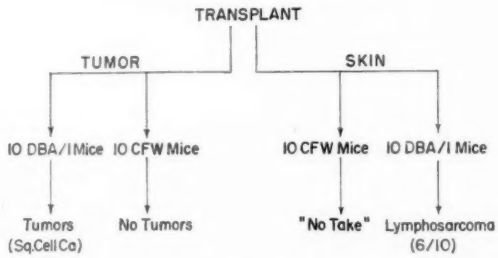

Fig. 2.—Induced squamous cell carcinoma in DBA/1 mice.

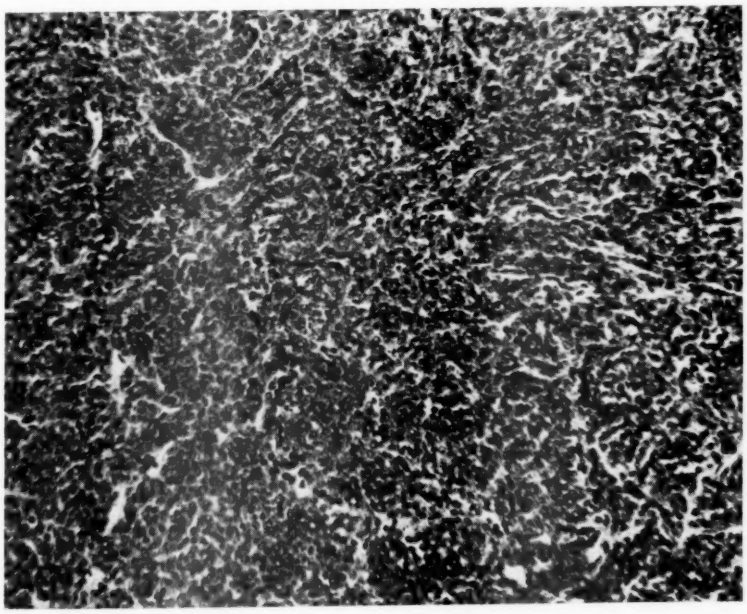

Fig. 3.—Lymphosarcoma arising from transplanted full-thickness skin graft.

of the skin grafts was normal for 10 days. Thereafter 6 of the 10 mice showed a beginning concentric growth around the periphery of the skin graft. Within 21 days the 6 mice showing this phenomenon died. This tumor growth was rapid, bulky in nature, and attained such a size that the mice could not stand or walk. The skin graft in the midst of this peripheral secondary tumor growth could still be recognized. Microscopic examination of this tumor was interpreted as a lymphosarcoma (Fig. 3). Sections of the skin graft at this time of transplantation showed a uniformly distributed line of cells in the subcutaneous layers which had the appearance of immature lymphocytes or lymphoblasts. Post-mortem examination of the animals with lymphosarcoma revealed no distant metastasis of the tumor. Homologous ventral surface skin grafts from the induced squamous cell cancer DBA/1 mice transplanted to 10 normal CFW mice resulted in the rejection of these skin grafts.

DISCUSSION

The macroscopic and histologic changes of cancerization to epithelial cells by a carcinogenic hydrocarbon are well known.[11, 12] The 3 following phases of induced carcinogenesis are recognizable.[1] There is the prewart or induction period where hyperplasia is the predominant response. Preneoplastic hyperplasia is thought to be highly specific since only carcinogenic irritants can produce it. Once this state has been induced, a benign tumor can be made to appear at that site. A tumor already present can have its rate of progressive growth hastened by the action of noncarcinogenic irritants. Preneoplastic hyperplasia must be biologically and morphologically distinct from ordinary hyperplasia of repair. The stages of the benign tumor are reflected by the appearance and growth of papillomas. The stage of cancer begins when the above benign induced tumor invades the subepithelial structures. Other phases than these classical stages may be present.

The mechanism of carcinogenesis may be partly the result of changes in the deeper tissues due to an interference with nutrition.[10] In vitro fibroblast cultures[8] exposed to methylcholanthrene results in a malignant change. The cancerous change does not result from an increased rate of cell division alone but a change in the ground substance. However, the rate of mitotic division was increased after long exposure to methylcholanthrene. These cells when injected into mice became malignant sarcomas.

Mouse skin when treated with a carcinogen, although it returns to a grossly normal-appearing state, has undergone a change which does not consist merely in the presence of a few latent cells.[13]

The inducement of skin cancers is related to dosage, time interval of application of the carcinogen, and the susceptibility of the animal.

Skin cancers can arise in response to a single application of a carcinogen.[4] A much smaller dosage of a carcinogen can induce a skin malignancy if applications are made at longer intervals of a month apart. Also an increasing dosage to large skin areas results in greater numbers of multiple skin cancers and shortens the time at which a cancer develops in a resistant strain of animals.[5]

Massive doses of methylcholanthrene produce malignant tumors more rapidly than smaller doses. The systemic effects noted in massive dosage are inflammation of the liver, kidneys, and lungs, hyperplasia of the bone marrow, spleen, and lymph nodes, and leukemia.[15]

It appears unlikely that methylcholanthrene or benzene was transferred with the skin grafts. Simpson and Cramer[14] studied the localization of methylcholanthrene in benzene solution when applied to skin by fluorescence. Solutions of this carcinogen in benzene gave a blue-violet fluorescence. By this method a dilution of one part of methylcholanthrene to a billion parts of benzene was detectable. A few seconds after painting the blue-violet fluorescence of the applied methylcholanthrene benzene solution changed to a yellow green indicating the solvent had evaporated and the dry carcinogen was present on the hair of the animal. After 6 to 10 days methylcholanthrene fluorescence disappeared from the skin. Questionable amounts of the applied carcinogen may be absorbed elsewhere but they must have been diluted or so altered since fluorescence was gone. Nearly all of the methylcholanthrene was accounted for but the above method in the sebaceous glands, lipids of the keratin layer, and the subcutaneous fat. Billingham and associates[2] used methylcholanthrene as a carcinogenic stimulus to induce squamous cell carcinomas in mice. A series of skin grafts were transferred to a recipient area of untreated skin of the same animal. The following observations were made: (1) Carcinogen-treated epidermis and thin split grafts of carcinogen-treated skin did not yield tumors. (2) Thick split grafts and full-thickness grafts of carcinogen-treated skin yielded a small number of tumors (5 to 30). (3) Tumors were observed in 13 of 21 transplants when a denuded carcinogen-treated area was resurfaced with untreated epidermis. (4) Reimplantation of a full-thickness graft of carcinogen-treated skin back into beds from which they were cut resulted in an increased rate and extent of carcinogenesis (2 to 6).

The inducement of lymphosarcoma resulting from the transplantation of skin grafts from animals treated with methylcholanthrene may be an expression of the systemic effect of the carcinogen. The effect of surgical trauma may also be an enhancing factor.[3, 7, 9] The appearance of abnormal lymphocytes or lymphoblasts in the subcutaneous layers of the skin grafts may be the mutated cells responsible for the lymphosarcoma.

In order to better understand these observations a series of experiments are underway. DBA/1 mice were painted with methylcholanthrene to produce squamous cell skin cancers. Cell-free serum, red blood cells, cellular extracts of ventral skin, and skins of varying thickness were taken from these painted animals and injected or transferred to normal DBA/1 mice.

SUMMARY

A purebred strain of DBA/1 mice painted with methylcholanthrene developed squamous cell skin carcinoma. The transfer of normal-appearing ventral skin to untreated DBA/1 mice resulted in lymphosarcoma. The theories and mechanisms responsible for these findings are reviewed.

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Discussion

DR. MARK M. RAVITCH (Baltimore, Md.).—What is the incidence of lymphosarcoma spontaneously in the same strain?

DR. HARDIN (Closing).—The strains of mice were obtained from Bar Harbor and, according to their information, there is no reportable incidence or, at present, it is very low or nonexistent; so this is again another fortunate circumstance of this experiment.

THE HETEROLOGOUS TRANSPLANTATION OF HUMAN CANCER IN UNTREATED SWISS-WEBSTER MICE

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INTRODUCTION

THE growth of human cancer in inbred strains of experimental animals would afford the investigator many new avenues of profitable research. Clearer definition under controlled conditions could be made, for example, of chemical mechanisms in abnormal human cell division, of environmental and nutritional factors in human cancer, of immunologic aspects of human cancer, of the induction of tolerance for human cancer, and of genetic aspects of human cancer. Studies on the hormonal alterations of neoplasia and chemotherapeutic investigations of cancer would be of even greater interest and significance if these studies could be conducted on human cancer growing under laboratory conditions.

Cancerous tissue, despite its inherent growth potentials, obeys the immunologic laws of tissue transplantation, and heterologous transplantation of human tissue into other animals invariably results in rejection of the heterograft within a few days. Suppression of the immune response or significant alteration of the immune response to tissue heterotransplants might result in acceptance of heterografts, and Toolan,[18] experimenting with this premise, was the first to achieve the growth of human cancer tissue in experimental animals. Total body irradiation or continued cortisone administration were the methods used to alter the response of the host, and in a series of 1,000 transplants Toolan[19] was able to grown human cancer in irradiated or cortisone-treated mice in 5 instances. Others working with this method have had great difficulty in obtaining successful human cancer transplants. Herbut,[10] for example, working with cortisone-treated and weanling mice was able to grow only 2 tumors in over 200 attempts, and both of these died out in subsequent generation transplants. The method is of great importance but has 2 major criticisms: (1) the incidence of successful tumor transplants is very low, and (2) the host, and conceivably the transplant, is and remains altered by suppression of its immunologic responses or by continued cortisone treatment.

This work was supported by a grant from the California Division of the American Cancer Society and by the Institutional Cancer Grant of the American Cancer Society.

Read at the Annual Meeting of the Society of University Surgeons, Boston, Mass., Feb. 13-15, 1958.

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In 1953 Medawar[14] introduced into the field of homologous tissue transplantation experiments designed to make the host tolerant of tissue homografts. He demonstrated that tolerance of a tissue homograft results when an animal has been confronted in fetal life with cells taken from its future donor or from some other member of the donor's inbred strain. The embryo is unable to respond to injected antigens by an immune reaction and this inability of an immune response continues into later life. This specific inhibition of response by acquainting or challenging the fetus with an antigen to which it will be later exposed again and to which it will remain tolerant in later life has been called "actively acquired tolerance." Later experiments by Medawar and Billingham[5] have proved that "acquired tolerance" is antigenically specific, not tissue specific, and that tolerance is due to an adaptation of the reactive mechanism of the host, not to an antigenic adaptation of the tolerated graft. Degrees of tolerance have been demonstrated, but it is due to a primary central failure of the mechanism of immunologic response. Great strides in the field of experimental homologous tissue transplantation have resulted from this brilliant discovery.

Two subsequent experiments in homologous transplantation of tissue of particular interest to this report were those of Billingham[2] and Weaver, Algire, and Prehn.[21] Billingham showed that a much simpler method for inducing tolerance of skin homografts in mice was the intravenous injection of newborn mice with spleen cells of the future donor. The orbital branch of the anterior facial vein of newborn mice was used or the sigmoidal sinus of the dura. Intravenous injection of newborn mice within 12 hours of birth with spleen cells of the future donor mouse induces actively acquired tolerance of the host to later skin homografts from the donor mouse in 95 per cent of the cases. The antigenic stimulus apparently reaches the newborn mouse in a "null period" during which it responds by tolerance rather than by immunity. This "null period" lasts only 1 or 2 days in the mouse, and varies with each animal species studied. Later work has strongly demonstrated that the immune response is mediated through lymphocytes and lymph-node cells of the host, and Weaver, Algire, and Prehn,[21] working on this hypothesis, have demonstrated that homologous cells will grow in vivo in diffusion chambers of such porosity that tissue fluids can diffuse through the walls of the diffusion chamber but host cells cannot.

These recent concepts in transplantation immunity led us to the experiments we are reporting on the transplantation of human cancer tissue in untreated inbred Swiss-Webster mice of a cancer-free strain.

EXPERIMENTS ON INDUCED TOLERANCE OF HETEROLOGOUS HUMAN CANCER TRANSPLANTS IN UNTREATED SWISS-WEBSTER MICE

Method.—The source of tumors for implantation was surgical specimens from the Stanford University operating rooms. As soon as the neoplasm was removed from the patient, a portion of it was taken aseptically and, in a nearby laboratory, immediately minced with fine scissors into fragments small enough to be implanted into the fetuses of pregnant mice of the Swiss-Webster strain. The anesthetized pregnant mouse was at the same time subjected to laparotomy and the minced viable aseptic tumor fragments were injected into the

exposed fetuses of the operated mouse. Concurrently, fragments of tumor tissue were implanted subcutaneously in the axilla or groin of the pregnant mother. This was accomplished by introducing the tumor fragment with a forceps through the open laparotomy incision. The laparotomy incision was closed and the mouse allowed to recover, awaiting the birth of the litter. In each instance some of the initial tumor specimen was quick-frozen, stored in a Deepfreeze at −79° C., and used for later implantation into the surviving members of the litter. Attempts were made to compare the success of intraembryonic injection of heterologous tumor tissue with other methods less likely to permit tolerance of the cancerous graft, and a group of animals was studied in which tumor tissue was implanted subcutaneously into newborn mice of Swiss-Webster strain or intracranially into adult Swiss-Webster mice. The number of animals and types of tumor transplanted intraembryonically into pregnant mice are listed in Table I, as well as the number of fetuses born that were reinjected on the fourth to sixth week of life. Subcutaneous implantation of tumor into newborn mice and intracranially into adult mice was also done in other animals, serving more or less as controls to the primary study.

TABLE I

A. SPECIMENS

Cheek	Nodes, neck, axilla	Sternum
Iliac	Craniopharyngioma	Bladder
Finger	Melanoma	Thyroid
Femur	Mediastinum	Kidney
Hand	Mandible	Hodgkin's
Ovary	Vertebrae	Vulva
Nose	Peritoneum	Brain
Leg	Gall bladder	Glioma
Breast	Sarcoma, elbow	Lung
Colon	Abdominal fluid	Bronchus
Rectum	Omentum	Parotid
Stomach	Adrenals	Abdomen

B. HUMAN TUMORS IMPLANTED INTRAEMBRYONICALLY INTO PREGNANT MICE

Number of human tumors	80
Number of operated pregnant mice	153
Number of fetuses born, later injected	470
Number of successful transplants in fetuses	0
Number of successful transplants in pregnant mother	4

Results.—Table II summarizes the results of our experience in attempting to grow human tumors by subcutaneous implantation into newborn mice. Table III summarizes the data on intracranial transplantation of human tumors into the brains of untreated Swiss-Webster mice. Tables II and III can be dismissed from further discussion since they merely demonstrate again that heterologous transplantation of human cancerous tissue into untreated mice, whether it be in newborn mice or intracranially in adult mice, is uniformly unsuccessful for practical purposes.

TABLE II. HUMAN TUMORS IMPLANTED SUBCUTANEOUSLY INTO NEWBORN MICE

Number of human tumors	23
Number of newborn mice	420
Number of successful transplants	0

TABLE III. HUMAN TUMORS TRANSPLANTED TO BRAINS OF SWISS-WEBSTER MICE

Number of human tumors	23
Number of mice transplanted	75
Number of successful transplants	2

The interesting and rather surprising results are evident in Table I. Briefly, of 80 human tumors injected intraembryonically in untreated Swiss-Webster mice, tolerance for subsequent heterologous implants of the initial tumors was not induced. However, 4 of the pregnant mothers developed human tumor transplants on the fortuitous simultaneous implantation of cancerous tissue in the axilla or groin at the time of the intraembryonic implantation.

The 4 human tumors that grew on heterologous implantation into pregnant mice were of diverse histologic structure. The most actively growing tumor was a squamous-cell carcinoma of the lung (H.T. 37) and the gross appearance of the viable implant is illustrated in Fig. 1. The other tumors that grew successfully were an epidermoid carcinoma of the skin (H.T. 36), a metastatic adenocarcinoma of the breast (H.T. 57), and a mediastinal tumor of mixed undifferentiated cell type (H.T. 64).

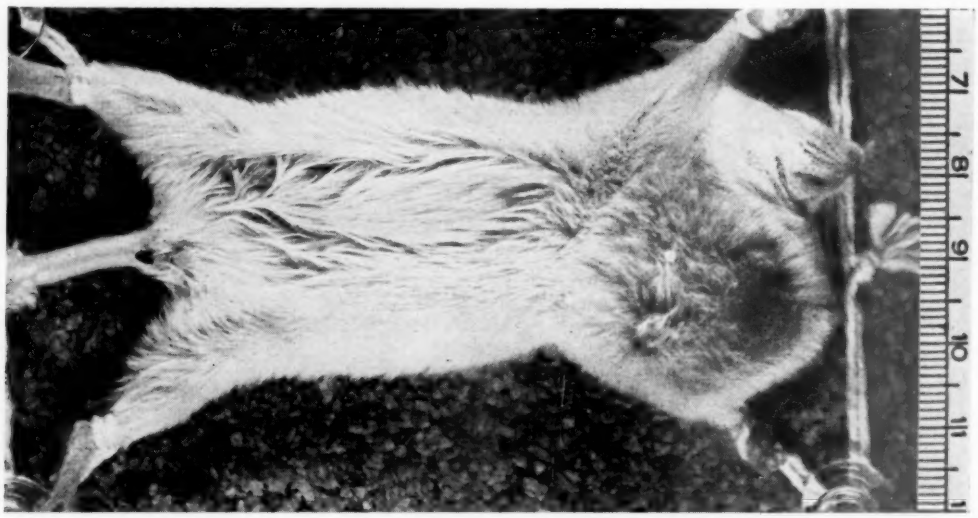
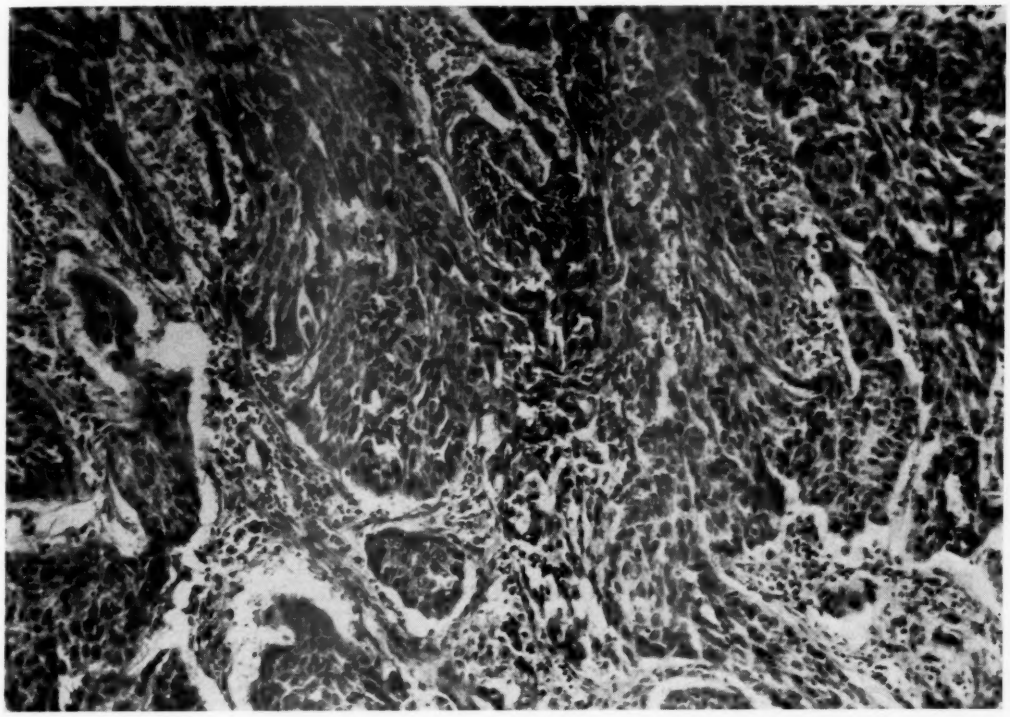


Fig. 1.—Mouse with viable tumor.

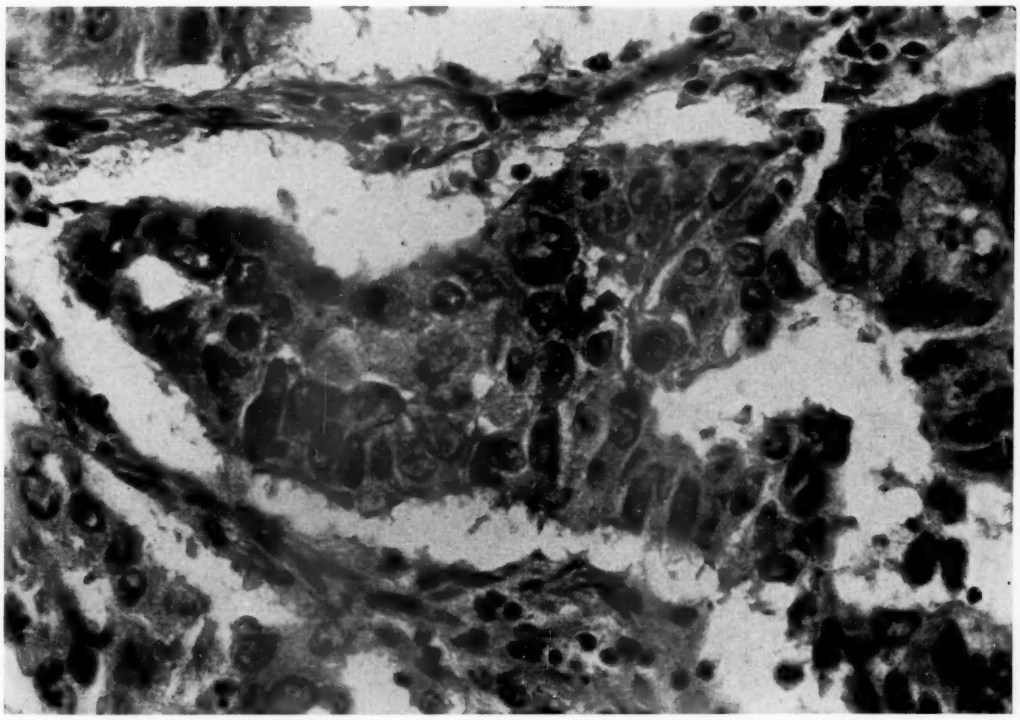
TABLE IV. FATE OF SUCCESSFUL TRANSPLANTATION IN SUBSEQUENT GENERATIONS

HUMAN TUMOR TYPE AND NUMBER	GENERATIONS								
	1	2	3	4	5	6	7	8	9
H.T. 37 Carcinoma bronchus Squamous cell Moderately differentiated	+	+	+	+	+	+	+	+	+
H.T. 36 Carcinoma of face Squamous cell Cheek	+	+	+	+					
H.T. 57 Adenocarcinoma of breast Metastatic	+	+	0	0	0	0			
H.T. 64 Mediastinal Tumor	+	0	0	0	0	0			

The subsequent fate of the 4 successful implants is seen in Table IV. The squamous-cell carcinoma of the bronchus is still growing successfully in the ninth generation transplant. The percentage of successful takes in each new generation implanted is still low, but is approximately 50 per cent of the animals injected. Nevertheless, the tumor is still growing and maintains histologic characteristics quite comparable with those of the initial tumor.

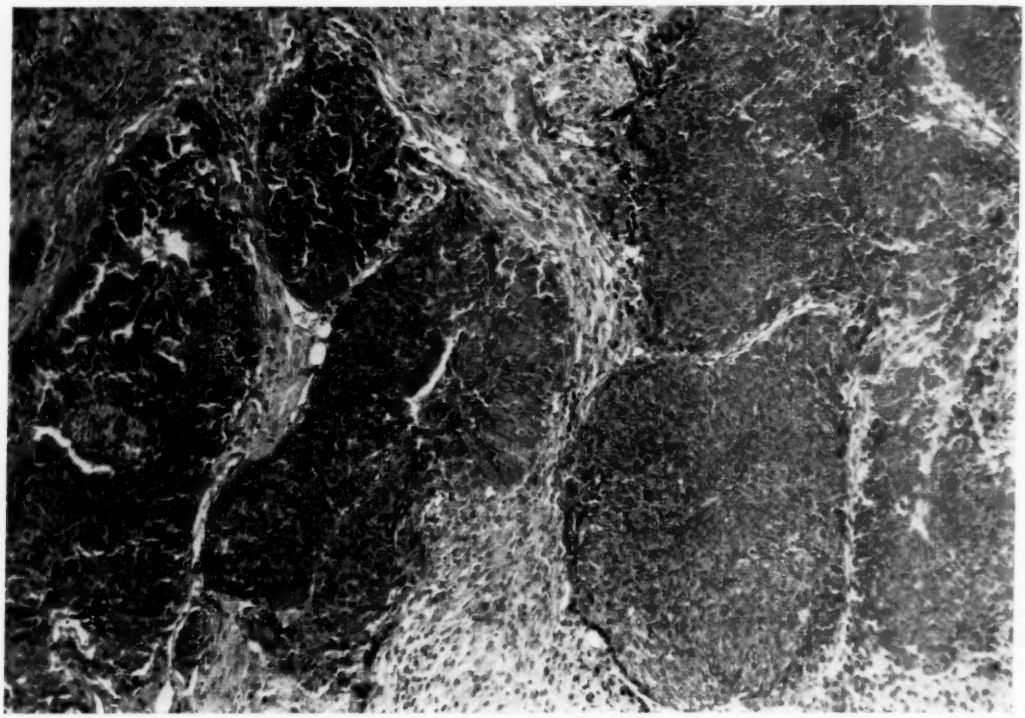


A.

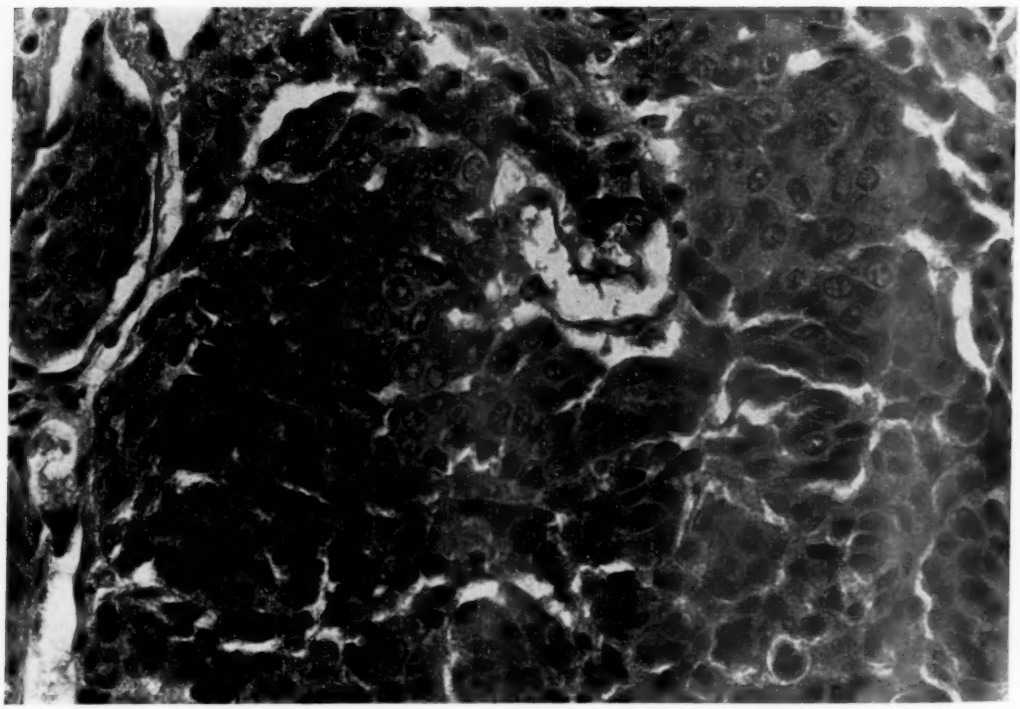


B.

Fig. 2.—Initial H.T. 37. *A*, Low power (×80). *B*, High power (×440).

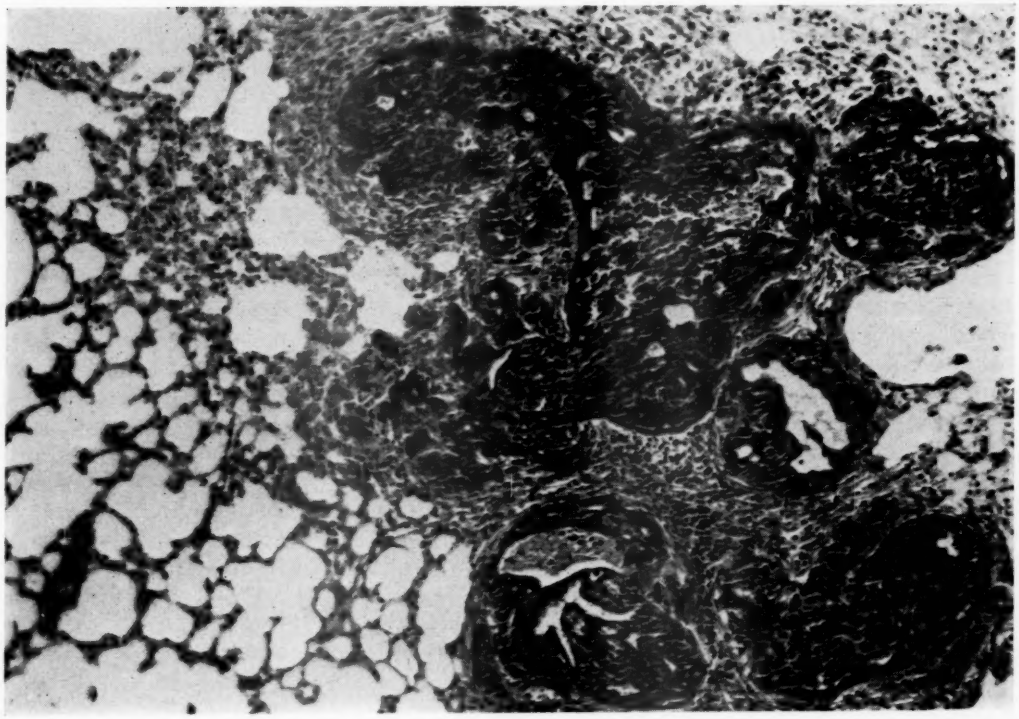


A.

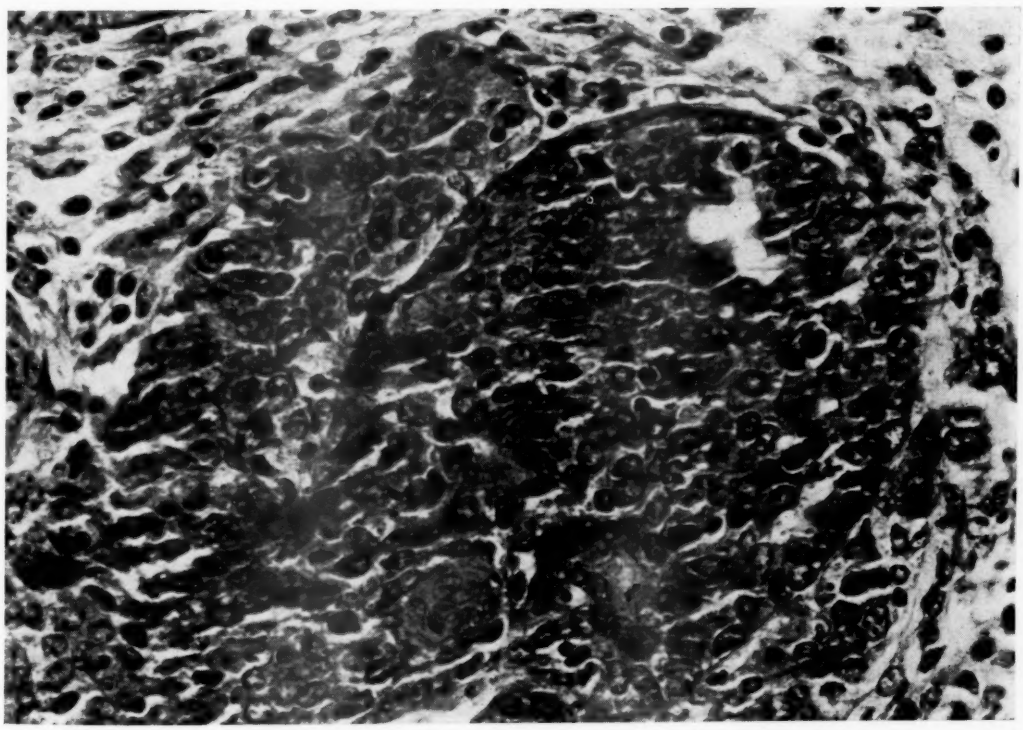


B.

Fig. 3.—Final H.T. 37—ninth generation. *A*, Low power (×112). *B*, High power (×440).



A.



B.

Fig. 4.—Lung metastases. *A*, Low power (×112). *B*, High power (×440).

Fig. 2 is initial histologic appearance. Fig. 3 is present histologic appearance in the ninth generation transplant. The tumors continue to behave in a cancerous fashion, grow continuously in size, eventually ulcerate and kill the animals, and occasionally produce systemic metastases in the lungs of the animals (Fig. 4).

The squamous-cell carcinoma of the cheek is still growing in the fourth generation transplant. The adenocarcinoma of the breast and the mediastinal tumor have not been serially transplantable in subsequent generations of the same strain of inbred mice.

EXPERIMENTS ON INTRAVENOUS INJECTION OF NEWBORN MICE

Method.—The technique of Billingham was slightly modified. Specimens of human cancer tissue were obtained from the operating room and taken immediately to the laboratory. The tissue was divided into three or more portions. The first portion was preserved for histologic study, the second was used in the intravenous injection, and the remainder was preserved in the Deepfreeze at −79° C. Tissues were handled under sterile conditions at all times.

The tissue to be used for injection was chopped into small pieces with scissors and forced through a No. 50 stainless steel mesh with a spatula. The resulting cellular suspension was washed 3 times with saline using a centrifuge speed of about 500 X G for 5 minutes. Of the final suspension, about 0.05 c.c. (300,000 to 500,000 cells per mm.^{3}) was injected into each Swiss-Webster mouse using the technique described by Billingham and Brent.[2] These injections were facilitated by wrapping the animals in paper towels and cooling them at −79° C. for 5 minutes prior to the injection.

When the injected mice reached 10 to 14 weeks of age, the cancer tissue which had been preserved in the Deepfreeze was removed and allowed to come to room temperature, and a portion of it was implanted subcutaneously in the adult mice. The areas of implantation were subsequently palpated, and 7 weeks after transplantation autopsies were performed.

Results.—The results of this experiment are given in Table V. In no instance, despite the successful intravenous injection of the newborn and young mice, were we able to obtain a successful transplantation of human tumor into the same strain of Swiss-Webster mice.

TABLE V

TISSUE	CONDITION	NUMBER OF ANIMALS AND AGE WHEN INJECTED	NUMBER OF ANIMALS AND AGE WHEN TRANSPLANTED	SUCCESSFUL GROWTH
Adenocarcinoma of colon	Fresh	8-1 day	6-13 weeks	0
Adenocarcinoma of colon	Fresh	10-1 day	6-12 weeks	0
Glioma of brain	Fresh	9-1 day	4-12 weeks	0
Urinary bladder tumor	Fresh	7-8 hours	6-10 weeks	0
Retroperitoneal undifferentiated tumor	Fresh	10-1 day	10-11 weeks	
		13-3 days	11-11 weeks	
		10-5 days	lost	
Melanocarcinoma, metastatic	Fresh	12-8 hours	9-10 weeks	0
	Fresh	10-4 hours	3-10 weeks	0
	Frozen	13-1 day	11-10 weeks	0
Adenocarcinoma of stomach	Frozen	9-2 days	8-13 weeks	0
Squamous-cell carcinoma of cheek	Frozen	6-3 days	5-14 weeks	0

EXPERIMENTS WITH MILLIPORE CAPSULE IMPLANTATION

Method.—The chamber employed was similar to that of Algire.[21] Lucite rings with outside diameter of 18 mm. and inside diameter of 13 mm. with a depth of 1.5 mm. were covered with millipore disks of pore size HA attached to the ring with adhesive solution. HA filters particles of size 0.5 μ or greater. The membranes were "painted" to the rings with adhesive solution of acetone containing 1 per cent dissolved Lucite. The chambers were sterilized by placing in 70 per cent alcohol for 15 minutes and were rinsed 3 times in distilled water.

Tissue was obtained fresh from the operating room and processed by 2 methods: (1) chunk and (2) suspension. In the chunk method tissue was chopped into small chunks, about 0.05 mm. cubes, and one chunk was transplanted in each chamber. In the suspension method, tissue was cut into small slivers and pressed through a No. 50 stainless steel mesh with a spatula. A saline suspension of tumor cell, approximately half, filled each chamber.

Swiss-Webster mice 8 to 10 weeks old were anesthetized with ether and chambers were implanted intraperitoneally or subcutaneously in the flank. Each tumor was implanted into 4 females and 2 males. Half of the animals received a suspension chamber; half received a chunk chamber. Animals were sacrificed 2 to 5 weeks following transplantation, and the chambers were opened for observation, gross and microscopic.

Results.—The results are listed in Table VI. Of over 50 animals containing 8 different tumors in millipore capsules, macroscopic and microscopic examination of the transplanted tissues revealed no evidence of growth. Chunks were cream-colored and cheesy in consistency. Chambers containing suspensions demonstrated no cellular proliferation. Microscopic examination showed homogeneous eosinophilic debris with no indication of the original cellular morphology. There was no indication of cellular infiltration from the host.

TABLE VI. MILLIPORE ENCAPSULATION

TISSUE	CONDITION	NUMBER OF ANIMALS INJECTED	SUCCESSFUL GROWTH
Adenocarcinoma of colon	Chunk	3	0
	Suspension	3	0
Adenocarcinoma of colon	Chunk	3	0
	Suspension	3	0
Glioma of brain	Chunk	3	0
	Suspension	3	0
Urinary bladder tumor	Chunk	3	0
	Suspension	3	0
Retroperitoneal undifferentiated tumor	Chunk	3	0
	Suspension	3	0
Melanocarcinoma, metastatic	Chunk	3	0
	Suspension	3	0
Adenocarcinoma of stomach	Chunk	3	0
	Suspension	3	0
Squamous-cell carcinoma of skin	Chunk	3	0
	Suspension	3	0
8 tumors	24 chunk		0
	24 suspension		0

DISCUSSION

Actively acquired tolerance introduces into immunology the concept of a specific inhibition of response and affords the surgeon exciting opportunities for the study of homologous transplantation of tissues. The induction of tolerance to heterologous tissue transplantation is far more difficult than for homologous tissue. It was hoped that if the heterologous tissue were cancerous that its inherent growth potential might be sufficiently strong to permit its survival on heterologous transplantation. The natural barriers to the growth of human cancer tissue in inbred strains of mice might be overcome by combining (1) the induction of tolerance with (2) the employment of tissue with potent inherent growth characteristics. The initial goal of our experiments based upon these concepts was to obtain the growth of human cancer in untreated Swiss-Webster mice, thereby making available human cancer in a controllable biologic environment for subsequent investigations. Interesting results have occurred which are rewarding but difficult of analysis in the light of present knowledge.

The discovery in 1953 of actively acquired tolerance was a brilliant new immunologic concept. It has been analyzed recently in detail by Billingham, Brent, and Medawar.[5] Tolerance of a tissue homograft results when an animal has been confronted in fetal life with cells taken from its future donor or from some member of the donor's inbred strain. It is the reverse of immunity to or rejection of a tissue homograft and depends upon (1) the inability of the fetus to respond to antigenic stimuli by becoming immunized and (2) the continued inability to do so after birth when challenged by the same antigenic stimuli. Tolerance is antigenically specific. Tolerance does not distinguish between the tissues of a given individual, but the stimulus which confers tolerance must be fully antigenic, that is, it must be one which would have elicited an immune response from an older animal. Cells such as erythrocytes are incapable of eliciting an immune response or of causing tolerance of tissue homografts. The white cells and nucleated spleen or tissue cells are capable of inducing tolerance, suggesting that the active chemical principle in the induction of tolerance is DNA nucleoprotein.

Every degree of tolerance is possible, ranging from a slight prolongation of survival time of homografted tissue beyond its normal median expectation in the new host to a complete permanent acceptance and incorporation of the homografted tissue into the host. The antigenic stimulus which causes embryos to become tolerant is one which stimulates an adult animal to become sensitive or immune, thereby precipitating homograft rejection. It might be suspected that this transition from one modality of response to another would occur gradually, and that there might be a neutral or "null period" either at or shortly after birth in which tolerance could still be induced. This, in fact, has proved to be true, and Billingham[2] has shown that the intravenous injection of splenic cells from a donor into a newborn host mouse via the anterior facial vein induces tolerance in almost 100 per cent of the animals, if the intravenous injection is done in the first 24 hours of life. Intravenous injection of 2-day-old mice produces tolerance in only 50 per cent of the animals, and intravenous injection of 5-day-old mice results in an immune response. This "null period" at and shortly after birth permitting a tolerant rather than an immune response has been shown to exist for mice, rats, and chicks. Its existence has simplified the induction of tolerance in laboratory animals and has greatly facilitated the study of the homograft and heterograft problem.

Tolerance can be abolished in mice by (1) injecting the tolerant mouse with cells from the regional lymph nodes of normal mice which have been actively immunized with the initial antigen or (2) injecting the tolerant mouse with cells from the lymph nodes of normal unimmunized mice. The former method produces a rapid restoration of immunologic reactivity, the latter a slower abolition of the tolerant state. This passive transfer of the immune response was first discovered by Mitchison[16] in 1953 and confirmed in detail by Billingham, Brent, and Medawar[4] in 1954. It has been called "adoptive immunization." It strongly suggests that the tolerated homograft continues to be a source of antigenic stimuli, and its susceptibility to an antigen-antibody response at a

peripheral level remains unchanged. Tolerance represents in all probability, therefore, a central failure of the mechanisms of immunologic reaction, rather than a local failure of immunity at the site of the graft.

Medawar[15] has recently related these diverse observations into a plausible hypothesis bearing on transplantation immunity and tolerance. The hypothesis is in two parts: (1) a continuous stream of desoxyribonucleic acid (DNA) nucleoprotein issues from most of the nucleated cells of the body toward the lymph nodes and spleen and this nucleoprotein or its nucleic acid moiety is incorporated into lymphocytes; and (2) one function of the lymphocyte in its life cycle is to restore nucleic acid to the peripheral tissues, thus completing the cycle. Leukocytes contain all antigens present in donor tissue and would be capable of inducing tolerance. The incorporation of the leukocytic transplanted nucleoproteins into the host at a time of life when the host is tolerant of antigenic stimuli would result in the incorporation of donor nucleoprotein into the regenerating and developing lymphocytes of the host. The host lymphocytes would serve as vectors to carry the incorporated nucleic acid and nucleoproteins to the tissues of the host, where again they would be utilized as the lymphocytes are destroyed in the peripheral tissues. The homograft reactions depend upon the interposition of the wrong kind of nucleic acid in a nucleic acid cycle. This hypothesis also fits in with other evidence, which will be discussed later, in which Weaver[21] has shown that the host's reaction against homografts is mediated by blood-borne cells. This hypothesis, however, does not conform with some of the observations herein reported on heterografted cancerous tissue, as will be discussed later.

Two other important questions require some discussion prior to directing our attention to an explanation of our results. These are: (1) does graft adaptation occur and (2) can secondary changes in the host result from excessive degrees of tolerance? The first question is still open to further experimentation; the latter seems to have been clearly answered by the appearance of "runt" disease or "homologous immune disease."

Billingham and his co-workers are quite convinced that graft adaptation does not occur. They cite experimental evidence that (1) a tolerated graft retains its power to provoke immunity when transplanted into a normal environment, (2) a tolerated graft succumbs to the immune reactions initiated by passive or "adoptive immunization," and (3) a fully tolerant host may be repeatedly grafted from its original donor. Cannon and his associates[6] still believe that graft adaptation does occur. They have experiments to demonstrate that tolerance varies with the age of donor and host. Very young homografts survive when the host and donor are one day of age, but homografts from the same donor applied to the same tolerant host a few weeks later may be rejected while the initial homograft continues to survive. Furthermore, the fact that tolerated homografts can be returned to the original donor with only 50 per cent success suggests that graft adaptation occurs. Crucial experiments to prove or disprove graft adaptation remain to be accomplished.

Working with different strains of inbred mice, Billingham[3] has shown that all degrees of tolerance occur. Some species of completely tolerant mice displayed gross involution of their lymph nodes, abnormal fibrotic spleens, progressive weight loss, and a short life span. This sequence of events has been called "runting" or "runt disease" by Billingham and suggests that in some species and under poorly-understood circumstances a graft-versus-host reaction is associated with tolerance. Similarly, in studies on tolerance in mice induced by total body irradiation, Trentin[20] found that transplantation of homologous bone marrow protected the animals initially from death, but that later some mice showed after a good initial recovery a progressive deterioration of hair growth, atrophy of lymphoid organs, body weight depression, general debility, and even death. This "late irradiation mortality" or "secondary irradiation syndrome" in tolerant animals seems similar to "runting" and has also been called "homologous immune disease" or "heterologous immune disease." Its origin in a graft-versus-host reaction seems most likely.

The antigens of tumor tissue and tolerance of tumor homografts have been studied by Billingham[5] and Koprowski.[13] The degree of tolerance which allows a tumor homograft to survive may be too low to sustain a skin homograft. Inherent growth potential of malignant cells may override to some degree the immune reactions of the host. The antigens of tumor tissue, however, are identical with those of skin, for tolerance of a tumor homograft also permits tolerance to homografted skin. A lower degree of tolerance suffices to allow a tumor homograft to grow than a skin homograft. Tumor homografts can have their tolerance abrogated by "adoptive immunization."[11]

Heterologous tolerance in mammals has been studied by Egdahl and his co-workers.[7, 8] Skin heterografts from rabbit to rat or mouse to rat become effectively vascularized for short periods but invariably die within 8 days. In a few animals temporary prolongation of heterograft survival up to 18 days was obtained by inducing tolerance by intraembryonic injection of rats with rabbit nucleated cells. Heterologous tolerance may be possible but very difficult to achieve.

Further light has been shed on these complex problems of tissue grafting by Algire,[1] employing a diffusion chamber technique for the study of cellular immunity. The diffusion chamber technique permits in vivo tissue culture. Cells or tissue placed within a porous chamber can be nourished by body fluids, of the host but separated from direct contact from the cells and tissues of the host. This requires the use of a diffusion chamber of such porosity that tissue fluids of the host may penetrate the chamber but cells of the host may not. The physical separation of the living graft from the host cells permits analysis of certain problems of host-graft relationships and reactions. Using this diffusion chamber technique Algire has shown that implants in contact with host cells are destroyed in 3 to 6 days, while grafts separated from host cells by the diffusion filter survive for the duration of the experiments (11 to 21 days). However, if transplanted or "target" cells are combined with immunized lymphoid cells within the millipore membrane chambers, such tissues are destroyed although

the immunized cells have been washed and the host has previously been unexposed to the target tissue antigen. Algire has concluded that filters block the destruction of foreign cells by an immune host and that antibodies cytotoxic to homografts are associated with cells, predominantly lymphocytes.

Unfortunately, the practical application of the diffusion chamber technique to permit survival and function of homologous or heterologous tissue has not been so rewarding. Sturgis[17] has reported the survival of ovarian homografts within millipore membrane chambers in ovariectomized rats, but histologic examination at 60 days showed complete degeneration of the follicles and ova. Egdahl and associates[8] have subsequently determined that in dogs and rabbits adrenocortical tissue placed within millipore chambers and implanted intraperitoneally will not survive or function. Similarly skin homografts and autografts maintained within intraperitoneal diffusion chambers for 2 weeks would not survive on retransplantation to the donor. The viability of the tissue within the millipore chamber for practical purposes is minimal and is inadequate for proper function, unless new evidence appears.

Our desire was to transplant human cancer into untreated inbred Swiss-Webster mice for purposes of controlled biologic studies. It was hoped that the combination of (1) the induction of acquired tolerance and (2) the inherent growth potential of cancerous tissue would enable us to disrupt the barriers to heterologous transplantation of tissue. Our approach followed 3 paths which have proved fruitful in the study of tissue transplantation: (1) the induction of actively acquired tolerance, (2) the intravenous injection of newborn animals, and (3) diffusion chamber techniques. Generally fresh viable cancer tissue was used for all aspects of the study, but frozen cancer tissue also had to be employed. The viability of this frozen cancer tissue has been proved by subsequent experiments and has also been demonstrated by Klein and co-workers.[12] The handling of the tumors seems sufficiently safe that this aspect of the work needs no further elaboration.

Actively induced tolerance has failed for us as a method of promoting growth of human cancer tissue heterologously in Swiss-Webster mice. The initial failure of the intrafetal injections to permit subsequent postnatal growth of heterologous tumor tissue might be explained by the inability of the injected tumor tissue to gain access to the circulation of the fetus, a process which might be essential for tolerance to occur. However, the subsequent experiments on the intravenous injection of newborn mice failed also to produce actively acquired tolerance, and we are forced to the conclusion that heterologous tumor tissue is so antigenically dissimilar to mouse tissue that tolerance cannot be induced, at least at the stages of mouse development investigated. The heteroantigen certainly reached the circulation of the newborn mouse at a time when tolerance would be expected. Our experiences with the practical applications of the diffusion chamber technique to the growth and study of heterologous cancer tissue has paralleled those of Sturgis[17] and Egdahl.[8] The tumor fragments may remain viable for short periods of time, but despite the fact we could not demonstrate penetration of the millipore chamber by cellular elements of the host the tumors failed to grow in these biologic culture chambers.

The growth of heterologous human cancer tissue in pregnant untreated Swiss-Webster mice is far more surprising than the failure to induce tolerance in the animals by the intraembryonic conditioning of the future host. This rewarding result occurred with 4 different tumors out of 80 studied, an incidence of 5 per cent. This frequency of tumor "takes" in the pregnant female mouse is far beyond that which would occur spontaneously in this strain of mice. Furthermore, this frequency of "takes" has not occurred in the newborn mouse nor in the control males which have been injected with human tumor in subsequent studies. The fact that the tumors resemble very closely the initial histologic appearance of the transplant or are identical with the initial transplant makes us believe that they are truly human tumors growing in untreated pregnant hosts. This surprisingly frequent growth of human cancer tissue on heterologous transplantation in untreated but pregnant mice raises the question of the relationship of pregnancy to immunity in tissue transplantation. We are at present studying the effects of repeated pregnancies on human tumor transplantation, and this will be reported at a future date. That graft adaptation has occurred or that the tumor itself possesses inherent qualities permitting heterologous transplantation is unlikely with the initial transplants, for these same tumors failed to grow in newborn mice or in intracranial locations. The frequency of tumor "takes" in pregnant untreated mice is as high as that reported in cortisone-treated or irradiated animals.

The subsequent fate of the 4 successful transplants is interesting. Two of the tumors failed to continue to grow on transplantation into successive generations of the same inbred strain of mice. The other two have continued to grow, one being in the fourth generation transplant, and the other (H.T. 37), a squamous-cell carcinoma of the lung, being in the ninth generation transplant. The percentage of "takes" is highest when the transplant is done into the litter of the tumor-bearing mother. "Takes" occur in both male and female offspring. The percentage of "takes" in each litter in the ninth generation transplants is 50 per cent. Our hopes, therefore, of obtaining a colony of untreated Swiss-Webster mice bearing human tumor transplants are high. Metastases from this tumor to the host lungs are not uncommon, and the tumor eventually kills the host. Several fundamental questions arise to be answered by further experiments. What permitted the initial "takes"? Is this truly human tumor? Could we have inadvertently induced a mouse tumor by the implantation of an unknown carcinogen or viral agent? Has a transformation of the original tumor occurred because of its growth in and subsequent passage through an unfamiliar resistant host? Can the tumor be retransplanted back into man? Is the effective propagating factor in the nucleus or the cytoplasm of the cancer cell? Has transformation of the host occurred so that it is now tolerant of human antigens? Does graft-versus-host reaction occur?

Our tentative concept of the answers to these questions that is most constant with present knowledge is as follows. (1) The initial "take" occurred because we transplanted highly malignant cells lacking "identity proteins" or "immunologic identity" into a heterologous host whose environment was altered by pregnancy to additionally favor heterologous tissue transplants. Green[9] has

reviewed the immunologic literature of cancer and has proposed a similar theory for cancer generally. "Antigenic loss is its *raison d'etre,* but the antigens that determine the specific nature of the tissue, and not the individual, are primarily lost." Also, "The more malignant a cell the more it lacks the substances which give it identity, first as a tissue, then as an individual, and last, may be to a slight extent, as a species." (2) The tumor is in all probability human tumor, but we have not absolutely proved this point. The microscopic appearance of the tumor remains closely similar to that of the initial transplant, even in the ninth generation transplants. Similarly the increasing but initially difficult frequency of "takes" in litters of oncoming generations suggests that the continued propagation of the tumor is following a human rather than a mouse pattern. Chromosome counts were considered but deemed unreliable in settling the question, for the chromosome counts of neoplastic tissue are so highly variable. Immunochemical studies of the transplanted tumor and growth of the transplanted tumor in tissue culture might yield a definite answer to this question, and Dr. Stanley's laboratories in Berkeley are helping us with these problems as well as seeking a viral agent which might be present in these transplants. (3) Antigenic transformation of the original tumor is undoubtedly occurring, as is incorporation of new antigens into the host mouse. This is manifested by the increasing tolerance of each successive generation of inbred mice to the tumor transplant. Both graft adaptation and host adaptation are occurring, for we are also beginning to have successful "takes" in males as well as unpregnant females in the eighth and ninth generations. (4) Retransplantation of the tumor back to the original donor would be extremely interesting. This retransplantation, if successful, would prove the human nature of the ninth generation tumor. Unfortunately, the patient has already succumbed from lung carcinoma. Retransplantation into other humans would not be entirely conclusive, unless the retransplanted tumor continued to grow indefinitely, since it would be apt to succumb in 5 to 14 days as might any homologous tissue transplant. The variables of graft adaptation and host adaptation would still be unknown in homologous transplants. We plan, however, to transplant human tissues into these tumor-tolerant mice to see if the incorporation of human antigens into inbred mice now permits other human antigenic tolerance. (5) Now that the tumor transplant is becoming firmly established, studies are contemplated in which efforts will be made to transplant separately total cells versus nuclei. This would answer the question as to whether the total cell is necessary for continued tumor transplantation. (6) The graft-versus-host reaction occurs in the sense that the tumor graft continues to grow, metastasizes, interferes with the nutrition of the animals, and eventually kills them.

It is quite obvious that we will have to turn to classic immunology, immunochemistry, immunogenetics, and genetics to answer problems resulting from these interesting experiences in making mice tolerant to human tumors. At the same time we are gratified to have this tumor available for chemotherapeutic and other studies in untreated Swiss-Webster mice. Proof of the human nature of this transplant awaits further verification.

CONCLUSION

We attempted to grow human tumor in untreated Swiss-Webster mice by employing the concepts of acquired tolerance, intravenous injection of the newborn mouse, and millipore chamber encapsulation of the heterotransplants. None of these methods permitted the growth of heterologous human cancer tissue in untreated animals.

However, the simultaneous implantation of human cancer tissue in pregnant mothers during the experiments on acquired tolerance resulted in 4 successful tumor "takes" of 80 different human tumors transplanted. Unfortunately, two of these, an adenocarcinoma of the breast and an undifferentiated mediastinal tumor, died out on transplantation into subsequent generations of mice. Fortunately, two of these, an epidermoid carcinoma of the skin and a squamous-cell carcinoma of the lung, are still growing in untreated animals on subsequent generation transplants. These tumors may be human transplants or may be induced tumors from other sources.

The squamous-cell carcinoma of the lung is now growing in the ninth generation transplant, and the incidence of "takes" is approximately 50 per cent. We have summarized evidence indicating that this is a human tumor to which this inbred strain of mice has become tolerant, but absolute proof of this point requires further immunochemical and genetic studies.

Further studies on the effects of pregnancy on tumor heterotransplantation, graft adaptation, host tolerance, and graft-versus-host reactions are under investigation.

We hope to have a satisfactory colony of untreated inbred mice tolerant to a human lung carcinoma in the not-too-distant future.

We are indebted to Paul E. Strandjord, one of our junior medical students, for his invaluable assistance in the conduct of the studies on intravenous injection of the newborn mice and millipore encapsulation studies.

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Discussion

DR. PETER V. MOULDER (Chicago, Ill.).—Do you think that these mothers are really Toolan animals or cortisone animals? Have you taken a similar type of one and treated it with corticoids or other things and then tried these human tumors?

DR. HOWARD ULFELDER (Boston, Mass.).—Have you done sex chromosome counts on the transplanted tumors? Was your lung tumor from a male? If you put it into female animals, you may be able to get a male sex chromosome count on the tumor. That is not too difficult, if it is not too undifferentiated.

Have you tried to induce tolerance in newborn or fetal animals to other tissues from the host? You can take the tumor, freeze it, put it away for a while, take the man from whom you took the tumor, and inject some blood cells or other cells into the embryos. Have you tried that to induce tolerance and then tried to transplant the tumor from the same donor?

DR. GARTH RUSSELL (Kansas City, Kan.).—There is fairly extensive work in normal tissue transplantation in the presence of pregnancy, particularly Dr. Elizabeth Sparrow's work in the rabbit, in which the survival time of skin homografts transplanted between unrelated rabbits is significantly extended. This is true, as it is in the case of cortisone, for the first-set response only. The second-set response is not affected, which is the rule with cortisone. Therefore, we feel that the corticoids are important in interfering with the setting up of the original sensitized response.

There is extensive work, particularly that of Dr. Hillary Koprowski, in the transplantation of mouse homologous tumors, not the heterologous system which he has used, in which there has been considerable success by injection of animals in utero with tumor cells, and the "later adaptation" of the tumor to the strain, untreated adult members of the strain. This has brought up one additional nuance and that is whether or not tumor tissues are indeed heterologous populations with some cells being genetically different from others. When one presents the animal with a sort of cafeteria type selection of these different cells, perhaps it selects them over a period of time which might account for the increasing incidence of takes over a period of time. I do not think we have enough evidence to speculate much further about that.

DR. MILTON T. EDGERTON (Baltimore, Md.).—This whole phenomenon of specific acquired tolerance, as pointed out so brilliantly by Medawar, has led to quite a wealth of investigation. It is interesting that this ability to make the newborn animal tolerant disappears very sharply, within a matter of days, usually, after birth.

The work of Dr. Richards would suggest that perhaps the ability of the newborn or the embryo animal to be made tolerant may not be just a matter of immunologic immaturity. The success of his 4 transplants into the pregnant mother of these animals raises again the question that this is more related to the changes that go on with pregnancy rather than to simple immaturity in the animal. The exciting thing about it is that it suggests here, with heterologous transplants, the possibility again of graft adaptation. We are beating our heads somewhat against a stone wall if, in order to get homografts to grow, we must severely knock out the adult animal to the point of making it a small gain. We have been a little encouraged in this line, even with adult tissue, in the animal, working with A-line and CBA mice, and getting some success in prolongation of adult homografts by exposing the grafts for short periods of time to a homologous host, withdrawing it, picking a new homologous host in the same strain and reapplying it. We all would like to see graft adaptation pushed.

DR. RICHARDS (Closing).—We have not tried putting the tumors into other strains of mice or into cortisone-treated mice, but certainly that is one step that we would like to carry out.

We were anxious in the beginning to get away from all host conditioning because we felt that whatever future avenue this work might take would be related to the initial conditioning of the host and that is the reason that we tried these various experiments in unconditioned animals.

The concept of graft adaptation that has been stressed by Dr. Edgerton is an interesting one and recently there has been more evidence brought to light that may make this possible. Some of you may have read about the conversion of French ducks to African ducks, and so on, by injecting DNA from one duck to another. This is a field which is certainly worthy of considerable future investigation.

STUDY OF ADJUVANT CANCER CHEMOTHERAPY BY MODEL EXPERIMENTS

With Comments on the National Clinical Adjuvant Chemotherapy Program

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STUDY of the limitations of surgery for malignancy reveals the following important factors: (1) There is little likelihood that there will be a significant improvement in the early diagnosis of most malignancies until simple, accurate, and fairly specific chemical tests of the presence of early tumors are discovered. (2) The structural limitations of local excisions are being approached. Extraordinary radical surgery is being accomplished successfully, but it seems reasonable to suggest that additional techniques will be proportionately less beneficial to the patient. (3) There is increasing evidence that some early cancers are systemic in distribution. Surgical excision and x-ray therapy can be effective only for the control of the local or regional spread of malignancy.

Recently there have been attempts to supplement excisional surgery with some form of systemic anticancer therapy. These include irradiation, such as the radioisotopes,[6] chemicals,[3] and factors related to host resistance.[9, 12] This paper concerns an evaluation of the benefits and dangers associated with the use of chemicals as adjuvants to excisional surgery.

In the laboratory it is difficult to simulate exactly the clinical conditions under which adjuvant therapy with anticancer agents is given. Transplanted tumors are biologically different from spontaneous tumors, and the use of transplantable cells and tissues even in special strains of mice is an artifice. Nevertheless separate elements of tumor growth can be studied by these experimental model systems. That the results of such animal studies cannot be directly applied to the clinical problem should be obvious.

METHODS

Ascites cell tumors consist of the growth and multiplication of single cancer cells as a suspension in abdominal ascites fluid which accumulates as the cell numbers increase. The ascites tumors used in this study include Krebs II, Ehrlich adenocarcinomas, and sarcoma-180.

Outbred Swiss mice were used in these studies; thus each experiment included a few mice with a "natural resistance" to the transplantable tumor being used. Such studies may, however, more closely approximate clinical conditions. A counted number of tumor cells were injected into a tail vein and subsequently a chemotherapeutic agent was given.

Read at the Annual Meeting of the Society of University Surgeons, Boston, Mass., Feb. 13-15, 1958.

Assay of the effect of the agent depended upon survival time, the number of animals with metastases, and the relative number of lung metastases which developed in control and experimental animals.

THE THERAPY OF UNESTABLISHED TUMOR CELLS

The relative effectiveness of anticancer agents against individual tumor cells in contrast to an approximate number of the same tumor cells organized as an established solid tumor has been studied by several investigators. Briefly, unestablished tumor cells usually are much more sensitive to chemotherapy although there is necessarily an approximation as to the number of cells in the solid tumors used for comparison. In general, those compounds which were curative against intravenously injected cancer cells also caused significant regression of the same tumor cells growing as a solid tumor. Rarely, however, could solid tumors be cured.

In some instances compounds which had little or no measurable effect upon the solid tumors did prevent the growth of the same cells injected intravenously.

The effect of triethylene thiophosphoramide (TSPA) upon Krebs tumor is an example of this finding (Table I). TSPA has cured no animals with the same cells growing as a solid tumor.

TABLE I

CELLS	SITE	NUMBER OF CELLS	AGENT	DOSAGE	ROUTE	TIME	+MICE/ TOTAL	LUNG METASTASES
Krebs	I.V.	1×10^6	TSPA	15 mg./Kg.	I.V.	-30 min.	2/14	3
Krebs	I.V.	1×10^6	———	control	———		11/14	56

Compounds which are sufficiently effective against a tumor that objective regressions can be attained will afford almost perfect protection against the same tumor cells injected intravenously. For example, actinomycin D given at maximum tolerated levels will eradicate some established solid tumors and will give almost perfect protection against sarcoma-180 cells given intravenously (Table II).

TABLE II

CELLS	SITE	NUMBER OF CELLS	AGENT	DOSAGE	ROUTE	TIME	+MICE/ TOTAL	LUNG METASTASES
Sarcoma-180	I.V.	250,000	Actinomycin D	1,000 mg./Kg.	I.V.	24 hr.	1/31	1
Sarcoma-180	I.V.	250,000	———	control	———		27/32	103

TABLE III

TUMOR	SITE	AGENT	DOSAGE	ROUTE	TIME	+MICE	LUNG METASTASES
Sarcoma-180	1×10^6	Actinomycin D	1,000 mg./Kg.	I.V.	-30 min.	1/30	3
Sarcoma-180	1×10^6	———	controls	———		12/26	104
Krebs II	1×10^6	Actinomycin D	1,000 mg./Kg.	I.V.	-30 min.	6/11	47/11
Krebs II	1×10^6	———	controls	———		8/11	43/11

SPECIFICITY OF ACTION

It is important to note that a compound effective against one tumor may be ineffective against another (Table III).

EFFECT OF DELAY IN GIVING CHEMOTHERAPY

From a practical standpoint it is important to determine how long after intravenous inoculation of the tumor cells the chemotherapeutic agent can be given and still protect the host. Again, the answer depends upon the tumor-host-agent relationship. If the tumor is very susceptible to the anticancer agent, effective protection is possible even after a 48- or 72-hour delay (Table IV).

TABLE IV. EFFECT OF ACTINOMYCIN D (1,000 MCG./KG.) INTRAVENOUSLY UPON 250,000 SARCOMA-180 CELLS GIVEN INTRAVENOUSLY

TIME	NUMBER OF MICE	NUMBER OF MICE WITH METASTASES	NUMBER OF LUNG METASTASES
½ hour	13*	1	3
Control	26	12	104
24 hours	17	0	0
Control	21	5	29
48 hours	13*	1	3
Control	21	8	59

*Lesser number of experimental mice reflects toxic deaths within 25 days after injection.

In contrast, if the tumor is less susceptible to the agent, a delay in therapy is very important. An example of such an experiment has been reported by Morales and associates.[11]

Unfortunately, there are so many variables in such studies that no one factor usually predominates. The lack of effectiveness of an agent given many hours subsequent to inoculation is inseparable from the following factors: (1) the host's ability to develop an "immune" response quickly, (2) effect of drug toxicity on host resistance, (3) the duration of action of the chemotherapeutic agent, (4) the multiplication rate of the tumor cells, and (5) inclusion of drug-resistant cells in the inoculum or the ability of the tumor cells to develop drug resistance quickly.

IMPORTANCE OF THE ROUTE OF DRUG ADMINISTRATION

Differences in the routes of drug administration may be important clinically and can be suitably studied in the laboratory. Several authors have demonstrated the spread of cancer cells throughout the body cavities and in surgical wounds.[10, 17] The proportion of such cells which are able to survive has not been accurately determined but clinical experience and autopsy findings confirm the seriousness of such dispersion. A number of investigators have recorded the cytotoxic effect of compounds injected directly into the peritoneal cavity containing ascites tumors.[5] In fact this technique is commonly employed as a sensitive preliminary screen of new chemotherapeutic agents.

It is logical to assume that if an agent is extremely unstable, such as methyl-bis (B-chloroethyl) amine hydrochloride (nitrogen mustard, HN_2), its

effectiveness would be reduced by any contact with biologic substances before reaching the tumor cells. This circumstance is demonstrated by the following experiment in which relatively "insensitive" tumor cells were used (Table V).

TABLE V. EFFECTIVENESS OF HN_2 GIVEN INTRAVENOUSLY OR INTRAPERITONEALLY UPON "INSENSITIVE" TUMOR CELLS INOCULATED INTO THE PERITONEAL CAVITY

(HN_2—3 mg./Kg.—24 hours after 500,000 Krebs cells given intraperitoneally to Swiss mice)

ROUTE OF TREATMENT	NUMBER OF MICE	SURVIVORS-DAYS AFTER THERAPY		
		10	20	30
Control	28	20	7	3
Intravenous	27	22	5	2
Intraperitoneal	28	23	16	11

The National Surgical Adjuvant Studies have been designed to minimize such effects. For example, the gastric and colon study protocols include fractional doses of triethylene thiophosphoramide at the time of surgery into the portal system, peritoneal cavity, and peripheral vascular system.

Cognizance of the import of tumor cell spread in surgical wounds by Smith[17] is evident from the number of investigators seeking cell-destroying compounds which can be used locally.[4] For this sort of localized tumor cell sterilization, relatively toxic compounds to the host can be used. Similarly it is hoped that nonabsorbable anticancer agents will be found which will be applicable to the pleural and peritoneal cavities and bowel lumen.

INFLUENCE OF THE NUMBER OF CANCER CELLS

As might be expected the size of the cell inoculum in relation to the amount of anticancer compound given is important. HN_2 will completely destroy small numbers of cancer cells which are resistant to it. As larger cell inocula are injected, the protection afforded by the drug becomes increasingly inadequate and survival of the experimental animals is reduced to that of the controls.

INFLUENCE OF THE SITE OF CANCER CELL IMPLANTATION, AGE, AND SEX OF THE HOST

The ability of cancer cells to grow at different sites varies enormously. For example, 1,000,000 Krebs II cells are necessary to produce metastases in 50 per cent of our strain of Swiss (male) mice when given intravenously; approximately 250,000 cells are needed for subcutaneous injection, less than 5,000 in the peritoneal cavity, and only 1 to 25 cells in the intracranial cavity. Prince[14] has published an excellent review of bioassay methods for detecting ascites cells and demonstration of the implantation frequency differences with various sites. That newborn mice are more susceptible to transplantation needs no emphasis.

When small numbers of ascites cells are used, even sex differences affect tumor establishment.

ADVERSE EFFECTS OF CHEMOTHERAPY

Disadvantages *directly* related to the administration of anticancer agents have been reported including local effects such as tumor necrosis with resultant

fistulas and hemorrhage, severe injury of the normal tissue cells which proliferate rapidly such as the bone marrow and epithelium of the gastrointestinal tract, and central nervous system toxicity with resultant nausea, vomiting, and profound anorexia. Additional toxic manifestations are specifically characteristic of individual compounds.

On the other hand, little has been stated concerning *indirect* adverse effects of anticancer agents.

Kondo[7] and a few other investigators have suggested that ineffective, toxic chemotherapeutic agents increase the possibility of metastases presumably by reducing host resistance. Confirmation of this thesis has been obtained by injecting short-acting toxic agents before injecting tumor cells in the blood stream. In addition, toxic doses of a compound injected into animals several weeks after inoculation with tumor cells seem to result in a greater number of tumor takes and lung metastases. These observations are important since some clinicians are using interrupted small doses of chemotherapeutic agents at monthly or greater intervals. It is difficult to imagine that this technique would affect the growth of established tumor metastases unless they were extremely sensitive to the drug.

HOST RESISTANCE FACTORS

Cortisone has been demonstrated to stimulate tumor growth and metastases of experimental tumors.[1, 13] Similar experiments in our laboratory have confirmed these findings (Table VI).

TABLE VI. COMPARISON OF THE NUMBER OF TUMOR CELLS TRANSPLANTABLE INTRAPERITONEALLY WITH AND WITHOUT CORTISONE

NUMBER OF CELLS	PRETREATMENT WITH CORTISONE (2.5 MG. × 3)	NO TREATMENT (+MICE/TOTAL)
10	0/8	0/8
100	3/3	0/8
1,000	6/7	0/8
10,000	7/7	3/8
(Autopsied in 30 days)		

CLINICAL OBSERVATIONS OF ADVERSE EFFECTS

Clinically, we have observed patients with advanced cancer deteriorate explosively after a course of ineffective therapy with alkylating agents or actinomycin D. Several patients with nonresponsive breast cancer have also shown a rapid growth of their metastases after cortisone therapy. Unfortunately, it has not been possible to observe comparable control groups. The validity of these informational experimental findings and clinical experiences with advanced malignancy cannot be applied directly to surgical adjuvant chemotherapy. However, these adverse effects upon the host must be kept in mind. They constitute a warning that only clinical studies with proper statistical design and subject to continuous review should be undertaken at this time.

THE EFFECT OF SURGERY

Physicians and laymen alike have pondered the possible adverse effects of surgery itself upon tumor growth. Much has been written on the subject but no adequately controlled studies have been made. There is little doubt that theoretically the stress of anesthesia and major surgery may reduce the host's resistance to malignancy and that exposing raw tissue planes and surfaces might allow rapid implantation of tumor cells. This speculation, however, must be tempered by recognition of the large number of patients who might not have the chance for curative or effective palliative surgery if exploratory operations are not performed. The frequency of clinical errors in diagnosis alone is adequate reason for insisting upon confirmation by tissue biopsy.

The magnitude of the effect of surgery upon tumor growth is undoubtedly related to the biology of the tumor, the extent of surgical procedure and anesthesia, and general condition of the patient.

One preliminary experiment is attached only as an illustration of the sort of experimental study which should be made (Table VII).

TABLE VII. THE EFFECT OF CELIOTOMY AND OMENTUMECTOMY UPON THE GROWTH OF TUMOR CELLS INJECTED INTRAVENOUSLY

(500,000 Krebs II cells injected intravenously followed by celiotomy in 2 to 5 hours)

	NUMBER OF MICE	+MICE	LUNG METASTASES	TAIL IMPLANTS
Experimental	14	12	130	2
Control	14	5	73	5

No significance is claimed for this experiment despite the adverse number of tail implants in the control animals which normally would be associated with an increased number of lung metastases. It is interesting that none of the mice developed tumor masses at the operative site. Direct injury to an abdomen containing ascites tumor cells usually results in prompt tumor growth at the injury site. In the present instance perhaps the tumor cells were cleared from the blood stream before the operation.

DISCUSSION OF EXPERIMENTAL RESULTS

The experiments used in this paper illustrate the variation in relationship of tumor, host, and chemotherapeutic agent. The selection of animals is important. Not only do strain differences exist but a tumor may grow much more rapidly in one sex. One must be careful to insure the stability of the ascites tumor throughout the experiments and to harvest the cells early so that the results are not skewed by the inclusion of clumps of cells which may be distributed evenly and which probably have a much better chance of surviving. Exact cell counts must be used rather than estimates. If a large number of mice are to be injected the cell suspension should be made up of solutions with proper glucose-electrolyte balance and immersed in ice water to maintain their viability. Only after all of the mice have been injected should they be assorted into the control and experimental groups. Finally, the intravenous injection

of the cancer cells into the tail vein must be done accurately and without undue pressure; otherwise implants will quickly develop at the site of vein injury. (It is usually well to warm the animals prior to inoculation so as to insure maximum dilatation of the tail veins.) Such implants commonly develop lung metastases presumably from repeated exfoliation of tumor cells. These animals must be discarded, particularly if the effect of a single injection of a chemotherapeutic agent is under study.

Properly the controls should be injected with an inactive but equally toxic substance, but this is not always feasible.

Evaluation by comparing the number of metastases visible on the lung surface could be a source of error if certain tumors tended not to grow in the periphery of the lung, lung adenomas were included, and fused nodules were miscounted. To obviate miscounts of fused nodules, a maximum of 10 metastases were credited to each lung segment (left, median, and right) even if more were present or pleural ascites had developed.

Another sort of experiment which more closely simulates the clinical problem has been described by Shapiro.[16] A transplanted tumor is allowed to grow to a prescribed size; then 80 per cent or total excision is done and a chemotherapeutic agent administered. In such an experiment utilizing the 755 tumors in mice and treatment with 6-mercaptopurine, there were no cures obtained in a group of animals with intact tumors, in those who had surgical excision of the mass alone, or in those treated by chemotherapy alone; but there were 47 per cent cures in a group having 80 per cent of the tumor removed and also given chemotherapy. Additional studies must be done with insensitive tumors in order to evaluate the limitations of this excellent technique. Again, one should be reminded that transplanted tumors are being used for these experiments and they should not be interpolated as indicative of the behavior of spontaneous tumors under similar conditions.

CLINICAL ADJUVANT CHEMOTHERAPY

We have previously indicated that surgical adjuvant chemotherapy has many connotations which differ from those associated with the palliative treatment of patients with advanced disease. These include removal of the gross tumor which otherwise may continue to exfoliate tumor cells, relatively unimpaired host resistance in early cases, a proportionately minute number of residual tumor cells to be destroyed by chemotherapy, and the practical advantage of giving the anticancer agent directly into the wound, body cavity, or regional vein (or even the lymphatics) if desirable.

At Roswell Park, 57 patients have been given triethylene thiophosphoramide or nitrogen mustard in conjunction with major surgery. In no instances has immediate death been associated with the adjuvant therapy. Two deaths from anastomosis failure and impaired wound healing in patients with advanced disease resulted; however, in the same period one control patient also died with a "nonhealing syndrome." Experimental studies in rats and dogs have failed to implicate the alkylating agents in bronchial stump and wound dehiscence even when fatal doses were used.[2, 15]

The toxic effects of alkylating agents on hematopoiesis were reflected by peripheral leukocyte counts below 3,000 in 8 patients and below 1,000 in one. Similarly platelet counts fell below 100,000 in 6 patients and below 50,000 in 4. Temporary lowering of reticulocyte counts was recorded for 5 to 10 days after which they were above normal.

The indirect dangers of adjuvant chemotherapy such as the theoretical possibility that a toxic ineffective drug might enhance tumor growth are still undetermined.

Individual clinics are studying adjuvant chemotherapy and utilizing a variety of drugs upon various tumors. None of us, however, have sufficient numbers of patients to conduct properly controlled studies in a reasonable length of time. Further, these patients will be exposed to the risk of similar serious complications without knowledge of the experience elsewhere. For these and other reasons a national program to coordinate surgical adjuvant cancer chemotherapy studies was established in 1956. These studies are listed in Table VIII.

TABLE VIII

TUMOR	AGENT	DATE BEGUN	CHAIRMAN	NUMBER OF CLINICS	ESTIMATED NUMBER OF PATIENTS YEARLY
Stomach	TSPA	July, 1957	W. Longmire	19	175
Lung	HN_2	July, 1957	A. Curreri	17	300
Colon	TSPA	August, 1957	W. Holden	8	130
Breast	TSPA	Organized	R. Noer	Open	
Ovary	Chlorambucil	Organized	J. Masterson	Open	

Independent but parallel adjuvant studies are being carried out by 20 surgery departments of Veterans Hospitals. Drs. Paul North, R. W. Dwight, and Felix Hughes are serving as advisors of these groups and Gilbert Beebe is the responsible statistician.

The exact details of dosage and routes of administration of the drug are omitted since it is felt that the present schedules are tentative.

The choice of agents was limited to those which had had clinical trial in patients with advanced disease, toxic effects which would not interfere with the repair of operative sites, and that had shown some effect upon established metastases. From a practical standpoint the gastric and colon studies do not fulfill these criteria since the alkylating agents have not caused regression in these tumors; however, since both of these malignancies are complicated by a high percentage of portal vein metastases and body cavity recurrences, it was felt that cautious evaluation of adjuvant therapy was justified.

Theoretically, effective destruction of carcinoma of the breast and ovary cells by adjuvant therapy with one of the alkylating agents should be extremely promising since these compounds have been shown capable of regressing an appreciable number of metastases.

This discussion should not be interpreted as depreciating individual adjuvant chemotherapy projects, but as a warning of the complications which

may ensue and the desirability of utilizing study programs with adequate statistical controls. The exploration of new drugs and radioactive compounds, combination treatments, and varied technique by individual experienced investigators will always be a required prelude to organized cooperative studies. The pioneer surgical adjuvant study in this country by Cole and his colleagues[3] is an excellent example.

DISCUSSION

There is no doubt that the available chemotherapeutic agents are relatively ineffective against established malignancy. Whether they will be more effective as adjuvants to excisional surgery remains unproved. The establishment of cooperative studies of cancer chemotherapy has the advantages of insuring adequate numbers of patients in a reasonable period of time, excellent statistical controls, exposure of a minimal number of patients to toxic drugs for evaluation, and stimulating surgeon's interest in anticancer agents and the techniques of executing cooperative clinical trials.

Too often a clinical impression that a drug or therapeutic technique is useful has resulted in a widespread demand for it by patients and physicians alike without regard for the validity of the supporting evidence or knowledge of its complications. Once a regime is established as having therapeutic efficacy, it becomes increasingly difficult to conduct unbiased clinical trials. For example, it is extremely difficult to find a group of surgeons who are willing (with the approval of their colleagues) to study therapeutic aspects of breast cancer. Categorical opinions as the effectiveness of preoperative x-ray, postoperative x-ray, quality and quantity of x-ray, a multitude of variations in surgical techniques, castration, hormone therapy, indications for surgery, and the importance of pathologic classifications made acceptance of a uniform protocol for a cooperative clinical trial extremely difficult.

Comparisons between malignancy and infection are trite, but the evolution of the therapy of osteomyelitis may provide a frame of reference for the present attempts to use a combination of chemotherapy and excisional surgery for cancer. Some years ago chronic osteomyelitis was a relatively common disease. It was treated by expectant medical care, surgical drainage, and débridement. With the advent of the first partially effective sulfanilamides, a combination of such drugs and less radical surgery was more successful. Finally, chronic osteomyelitis as a surgical problem has practically disappeared with the preventive and early use of effective antibiotics; rarely is extensive surgery necessary. It is interesting to note in those instances in which drug resistance has occurred attempts are being made to bolster host resistance by providing "hyperimmunity."

Sanguine speculation about the future of cancer chemotherapy is difficult. The first few staggering steps toward the application of these agents to solid tumors have been discouraging. The history of cancer chemotherapy may parallel that of chronic osteomyelitis; however, it would seem that a much longer period of time will lapse before comparable effective therapy will be available.

CONCLUSIONS

1. Experimental studies of various aspects of adjuvant cancer chemotherapy resulted in the following conclusions: (a) Numbers of certain individual, unestablished tumor cells can be completely destroyed by anticancer agents, whereas control of their growth when established is not possible. (b) The activity of a chemotherapeutic compound on a tumor is specific; it may not be effective against related tumors. (c) Effective compounds will be curative even when the administration is delayed following tumor cell inoculations or when given by several different injection routes. (d) Relatively ineffective compounds must be given soon after tumor cell inoculation and by a direct injection route. (e) The size of a tumor cell inoculum in relation to the dosage and effectiveness of the anticancer agent determines the completeness of tumor growth prevention. (f) Toxic ineffective chemotherapeutic agents may enhance tumor growth—perhaps by depression of "host resistance." (g) Observations that cortisone may enhance the growth of experimental tumors were verified. (h) A preliminary experiment indicates that the stress of anesthesia and surgery may enhance tumor growth.

2. Surgical adjuvant chemotherapy has been used in 57 patients without causing serious postoperative complications. The toxic effects of the alkylating agents which were used resulted in a depression of peripheral leukocytes below 3,000 in 8 patients and below 1,000 in one patient. Similarly, platelet counts fell below 100,000 in 6 patients and below 50,000 in 4 patients.

3. The organization of a national program of cooperative surgical adjuvant cancer chemotherapy study groups was reviewed.

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Discussion

DR. HENRY T. RANDALL (New York, N. Y.).—Is there today a substance which is worthwhile in practice to use in irrigating a wound or put locally into a wound which is not likely to cause trouble and, at the same time, may have some efficacy in reducing the danger of local implantation?

DR. EDWARD L. HOWES (New York, N. Y.).—I would like to ask the source of your actinomycin D. Did you grow it yourself from actinomycin or are you using the Italian actinomycin D for this purpose?

There is also a Clorpactin solution that kills local cancer cells and it might escape the uninitiated that this is a Dakin's solution, which brings up the point I want to make. Free cancer cells are killed easily with iodine, with a hammer, with anything. This is not the problem.

The problem is to take care of the cancer cells that are attached to a blood supply. This is the difficult thing. It is easy to kill the free cancer cells either in the lumen of the bowel or in wounds by simply washing them out with some hypertonic solution or some solution that is alkaline, like atraphonolamine, for example, or propylene glycol, a 25 per cent solution. This problem of using solutions in wounds or locally or in the large bowel to get rid of the free cells is not difficult, but when you deal with cell aggregates, you have quite a different problem in which it is very difficult to get perforation of your media to get inside of the aggregate to kill it.

One thinks immediately of using surface tension-reducing substances that would cause the substance to go further and penetrate into the cell aggregate, but unfortunately this does not work.

DR. MOORE (Closing).—These questions are, of course, pertinent. It is much easier to kill single cells, small clumps of cells, and cells that are not attached. But that is just the point. We have not been doing this and they have been killing our patients. For example, 15 per cent of all patients with anterior resections of the colon are presumed to die of recurrences in the line of resection. Many of these deaths are caused by cells left in the lumen. Nothing much has been done about it until the last few years.

Almost 40 per cent of recurrences of head and neck tumors are in the area of the wound and many of these are recurrences from the spread of tumor cells in the area. They have not been taken care of either. It is correct that Clorpactin is very similar to Dakin's solution and Dakin's solution does work almost as well. Iodine solutions work, too, and dilute formalin works fairly well but do not misplace the decimal point in making up the solution.

There are compounds coming along that are crude at present, but they will be very important to all of us in a few years. It is important that we study them.

As far as the source of actinomycin is concerned, there are actinomycins A through M. I presume you mean actinomycin D. It is available from Merck. It is the only chemical, so far as I know, that has affected adenocarcinomas of the gastrointestinal tract.

THE USE OF A TOTALLY OCCLUSIVE PUMP AS A FLOWMETER WITH OBSERVATIONS ON HEMOLYSIS CAUSED BY OCCLUSIVE AND NONOCCLUSIVE PUMPS AND OTHER PUMP-OXYGENATOR COMPONENTS

JAMES S. MCCAUGHAN, JR., M.D.* (BY INVITATION), HARRISON MCMICHAEL, M.D.** (BY INVITATION), JOHN C. SCHUDER, PH.D. (BY INVITATION), AND CHARLES K. KIRBY, M.D., PHILADELPHIA, PA.

(From the Harrison Department of Surgical Research, School of Medicine, University of Pennsylvania)

THESE experiments were undertaken to determine whether or not a totally occlusive DeBakey pump would produce linear flow rate curves independent of resistance and to measure the hemolysis caused by occlusive and nonocclusive pumps at various flow rates. There is a wide difference of opinion concerning these fundamental problems and also concerning the contribution of various oxygenator parts to hemolysis. We were not able to reconcile these differences of opinion by analysis of the work reported by others and concluded that additional studies in our laboratory might be helpful in clarifying these problems.

The usual method of reporting hemolysis in milligrams of free hemoglobin per 100 c.c. of plasma is inherently subject to errors in interpretation because of variations in the blood volume and hematocrit. We, therefore, devised a method of calculating free hemoglobin which included suitable corrections for the dilution factors.

With the ever-increasing use of extracorporeal circulation of various types, potential abnormalities of blood elements produced by pumps, exposure to air, turbulence, and so forth have become increasingly important. Hemolysis is apparently not harmful until very high levels of plasma hemoglobin are reached, but measurement of the amount is relatively simple and, largely for this reason, has been used as an index of the damage being done to the blood as a whole, although a clear-cut correlation with injury to other blood elements has not been demonstrated.

USE OF A TOTALLY OCCLUSIVE DE BAKEY PUMP AS A FLOWMETER

A system consisting of a DeBakey pump totally occluding ½ inch rubber tubing, ½ inch Mayon† tubing, and 2 reservoirs was set up as shown in Figs. 1 and 2. The height of the water level above the floor (Z_1) and the height of the discharge end of the system above the floor (Z_2) were noted.

Supported by U.S.P.H.S. Grant 2331C, The John A. Hartford Foundation, Inc., and the York County (Pa.) Heart Association.

Read at the Annual Meeting of the Society of University Surgeons, Boston, Mass., Feb. 13-15, 1958.

*Research Fellow, Heart Association of Southeastern Pennsylvania.

**Research Fellow, United States Public Health Service.

†Mayon Plastics, 17 Ave. North, Hopkins, Minn.

Water was pumped through the system at different rates and each rate was determined by measuring the amount pumped per unit of time. The pump speed was determined by actually counting the number of revolutions during a given time interval. The height of the discharge end was then varied and the flow rate was measured at each level. The same procedure was repeated with catheters of varying size and different heights of the inlet level (Z_1). The various flow rates were then plotted as a function of the number of revolutions per minute of the pump (Fig. 3).

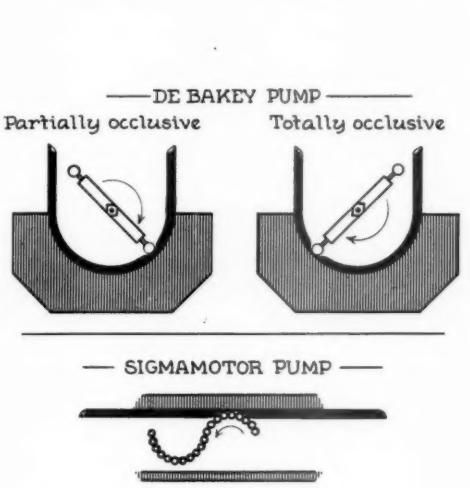

Fig. 1.

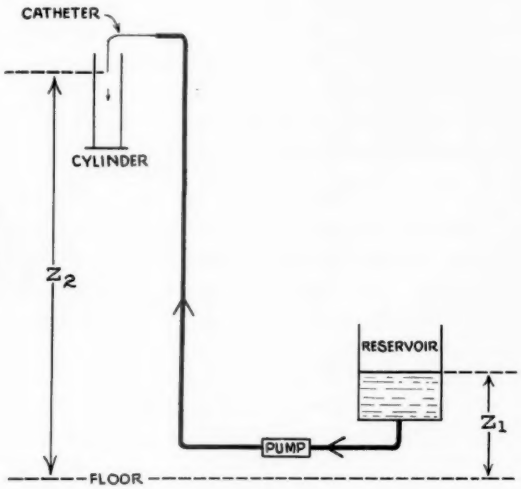

Fig. 2.

Fig. 1.—The difference between partial and total occlusion of the tubing in the pumps is shown diagrammatically.

Fig. 2.—This illustrates the circuit used to vary the pressure head for measuring its effect on flow rate.

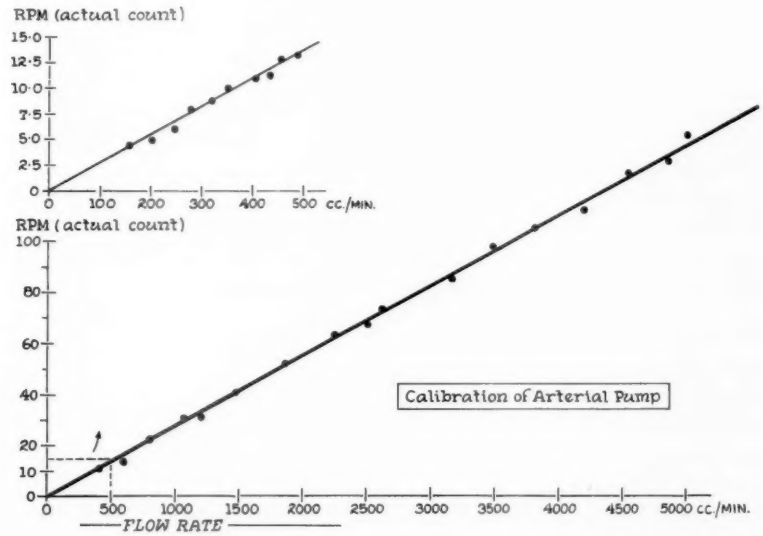

Fig. 3.—The flow was directly proportional to the speed of the pump in revolutions per minute. The inset shows the data for low flow rates.

Results.—Fig. 3 shows that the flow rate was independent of the pressure against which the pump was working over a measured range of 10 to 600 mm. Hg. Therefore, the flow rate is independent of the patient's blood pressure, of the distance between the patient and the pump, and of the size of the catheter. Every time the pump arm makes a complete revolution, a constant quantity of fluid is discharged. This quantity is dependent

only upon the length of the tubing in the pump head and the inner diameter of the tubing. After calibrating the pump, the precise flow rate can be determined by counting the number of revolutions per minute. This is not true of nonocclusive pumps,[1, 9] whose flow rate may vary markedly with changing blood pressure, with small changes in the height of the operating table, and with the size of the catheter.

We have found it necessary to use rubber tubing in the pump head because total occlusion causes splitting of plastic tubing. If the discharge end of a totally occlusive pump is completely obstructed, the pump will continue to force fluid into the tubing until the increasing pressure causes disruption, usually at a connector. To prevent this, we have incorporated an automatic pressure stop in the arterial line. If the pressure rises to a dangerous level this device automatically stops the arterial pump and makes a loud buzzing noise. When the condition is corrected and the pressure decreases to a safe level, the pump automatically starts again.

HEMOLYSIS

Hemolysis has usually been recorded in milligrams per cent of free hemoglobin in the plasma. However, wide variations in the blood volume and hematocrit may cause errors unless they are taken into account. For example, if 500 c.c. of blood with a hematocrit of 40 per cent is run through a closed circuit, the hemoglobin released will be diluted in 300 c.c. of plasma. However, if 1,000 c.c. of this blood is tested under the same conditions, the plasma hemoglobin will be diluted in 600 c.c. of plasma. If the results are reported in milligrams per cent of plasma hemoglobin, the hemolysis will appear to be only one-half as great in the second system as in the first—although it is actually the same.

Therefore in the following experiments we have determined the milligrams of hemoglobin formed per 100 c.c. of plasma and multiplied this by the volume of plasma to find the actual amount of free hemoglobin in the system at that time. This value is independent of the blood volume and is therefore much more accurate for comparative purposes.

METHOD OF DETERMINATION OF PLASMA HEMOGLOBIN

A known quantity of blood was put into the system being tested and the hematocrit was determined. At noted time intervals 8 c.c. samples were removed and centrifuged for 30 minutes, and the plasma was removed. One cubic centimeter of the plasma was then added to 5 c.c. of distilled water, and one drop of concentrated ammonia was added just prior to reading the optical density of the solution on a Beckman Quartz Spectrophotometer. The time interval is important since after 10 minutes the ammonia will induce the formation of hematin, which may cause erroneous readings. A wave length of 540 millimicra and a slit width of 0.025 mm. were used. The optical densities were converted to milligrams of hemoglobin per 100 c.c. of plasma by multiplying them by the conversion factor obtained from a calibration curve of known hemoglobin concentrations.[4, 5, 7]

Sample Calculation.—

Blood volume = 3,380 c.c.
Hematocrit = 34 per cent
Plasma volume = 2,230 c.c.
Optical density × conversion factor = milligrams hemoglobin per 100 c.c. of plasma
$0.136 \times 528.9 = 72$
Milligrams per cent plasma hemoglobin × plasma volume = milligrams of hemoglobin
$72/100 \times 2{,}230 = 1{,}610$

COMPARISON OF HEMOLYSIS CAUSED BY A SIGMAMOTOR PUMP AND A DE BAKEY PUMP

A closed system consisting of ½ inch rubber tubing in the pump head, ½ inch Mayon tubing, 2 stainless steel ½-½ inch connectors, and a Lucite reservoir was used with the flow produced first by a Sigmamotor* finger pump and then by a totally occlusive DeBakey

*Model T-6S, Sigmamotor Co., Middleport, N. Y.

pump* (Figs. 1 and 10, *E*). Pooled 7-day-old citrated canine blood (1,300 c.c.) was circulated at varying known flow rates with each pump. The Sigmamotor pump was adjusted so that it just occluded the rubber tubing. During each run several samples were taken at noted time intervals for the determination of the free hemoglobin present in the plasma. These values were then plotted as a function of time and the rate of release of hemoglobin (miiligrams per hour) was determined for each flow rate from the slopes of the curves (Figs. 4 and 5). The rate of formation of plasma hemoglobin (and therefore the rate of erythrocyte destruction) was then plotted for each flow rate for each pump.

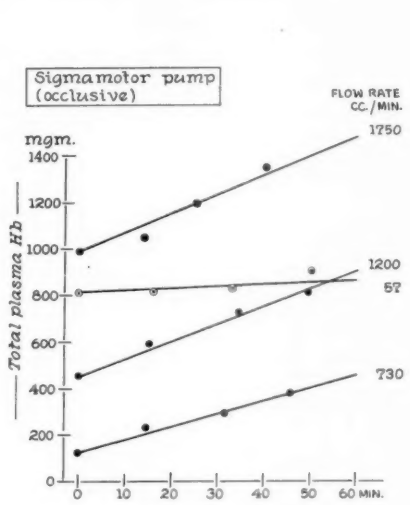

Fig. 4.

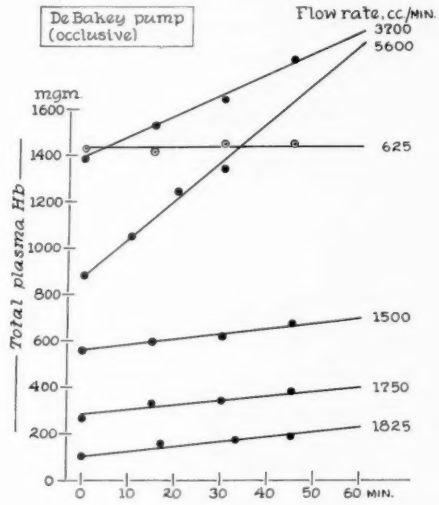

Fig. 5.

Figs. 4 and 5.—The total plasma hemoglobin in the system is plotted as a function of time and each curve represents a different flow rate. The rate of hemolysis can therefore be determined from the slope of each curve.

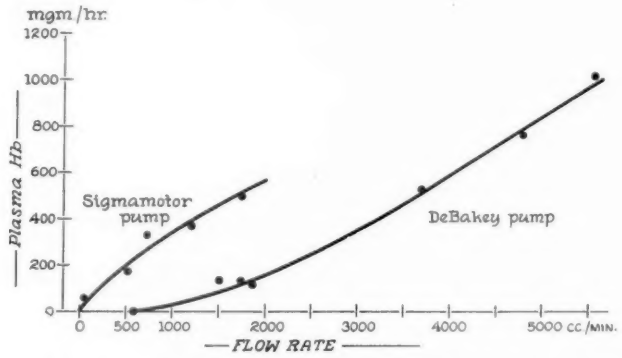

Fig. 6.—The rate of hemolysis at different flow rates caused by a Sigmamotor pump is compared with a totally occlusive DeBakey pump.

Results.—Fig. 6 shows that the Sigmamotor pump caused several times more hemolysis than the DeBakey pump when operating under identical conditions and at the same flow rates.

THE EFFECT OF TOTAL OCCLUSION OF A DE BAKEY PUMP ON HEMOLYSIS

Six-day-old citrated canine blood (1,200 c.c.) was circulated through a closed circuit similar to that described above. A totally occlusive DeBakey pump operating at a speed of 94 r.p.m. produced a flow rate of 3,600 c.c. per minute and samples were taken to determine the rate of hemolysis (Fig. 7). The length of the pump arms were then shortened

*Mark Co., Randolph, Mass.

so that there was only partial occlusion and the speed of the pump was increased until the flow rate was again 3,600 c.c. per minute. The rate of hemolysis was again determined. In this manner the degree of occlusion was varied several times and the number of revolutions per minute required to produce a flow of 3,600 c.c. per minute was noted. The ratio of the r.p.m. required with partial occlusion to the r.p.m. required with total occlusion to circulate 3,600 c.c. per minute was used to express the degree of occlusion. The rate of hemolysis was then plotted as a function of the degree of occlusion.

Results.—Fig. 8 shows that total occlusion of the pump caused more hemolysis than partial occlusion. The erythrocyte damage decreased with decreasing occlusion until a minimum value was reached. In order to reduce the rate of hemolysis significantly, the pump must be made sufficiently nonocclusive and not merely nonocclusive.

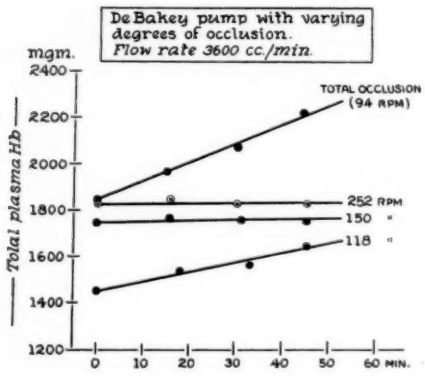

Fig. 7.

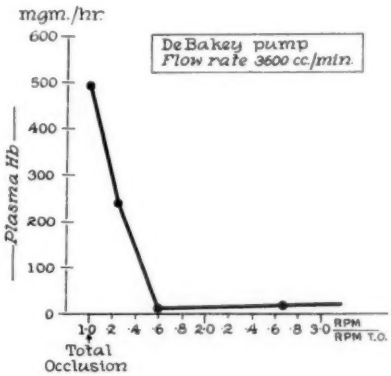

Fig. 8.

Fig. 7.—The plasma hemoglobin is plotted as a function of time for different degrees of occlusion of a DeBakey pump at speeds producing a low rate of 3,600 c.c. per minute.

Fig. 8.—The rate of hemolysis is plotted against the degree of occlusion which is represented by the ratio of the speed in r.p.m. required with partial occlusion to the r.p.m. required with total occlusion. (r.p.m./r.p.m.t.o.).

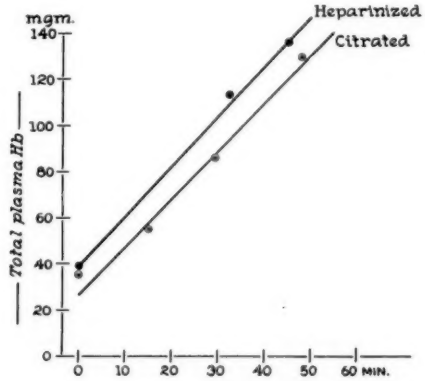

Fig. 9.—The rate of hemolysis of citrated blood is compared with heparinized blood at a flow rate of 1,750 c.c. per minute.

COMPARISON OF ANTICOAGULANT SOLUTIONS (HEPARIN AND A.C.D.)

Fresh canine blood (500 c.c.) was mixed with 18 mg. of heparin in 25 c.c. of saline. Another 500 c.c. of the blood was mixed with 120 c.c. of A.C.D. solution. Both were circulated through a totally occlusive DeBakey pump at 1,750 c.c. per minute and the rate of hemoglobin release was measured.

Results.—Fig. 9 shows that there was no appreciable difference in the rate of free hemoglobin formation in fresh citrated (124 mg. per hour) and fresh heparinized (128 mg. per hour) blood.

CONTRIBUTION OF THE COMPONENTS OF A SCREEN OXYGENATOR TO HEMOLYSIS

A stationary vertical screen Gibbon-type oxygenator* was assembled as shown diagrammatically in Fig. 10, *A*. A No. 20 plastic catheter was placed inside a ⅜ inch Mayon tube located between the ½ inch Mayon arterial and venous lines to simulate the arterial catheter used clinically. The DeBakey arterial pump was operated with total occlusion of ½ inch rubber tubing at a flow rate of 3,000 c.c. per minute but the recirculation pump was only partially occlusive. Heparinized human blood (2,500 c.c.) drawn 18 hours previously was circulated through the oxygenator, a filter,† and the arterial catheter, then back into the priming reservoir. Ten liters per minute of a 95 per cent oxygen-5 per cent carbon dioxide mixture were admitted to the oxygenator case. The pressure at the discharge end of the arterial pump was approximately 470 mm. Hg.

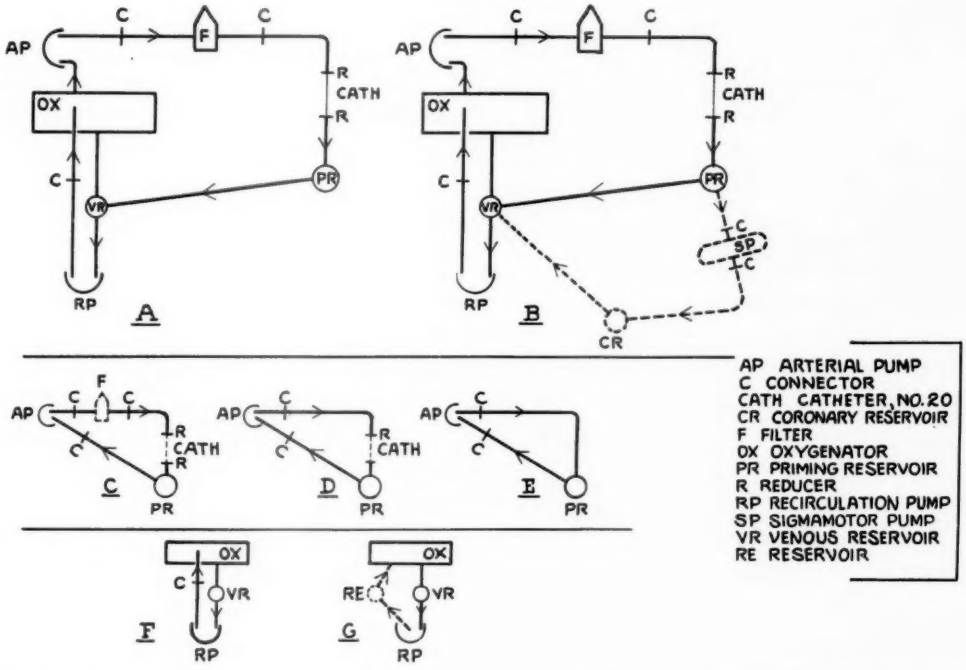

Fig. 10.—These are circuit diagrams of the extracorporeal circuits used to determine the contribution of each component to hemolysis. They are described fully in the text.

The blood was diluted with 580 c.c. of saline from the filming process which was retained to prevent blood loss so that the exact blood volume would be known. Samples were taken periodically and the rate of plasma hemoglobin formation was determined (Fig. 11).

To evaluate the effect of intracardiac suction, the experiment was repeated while blood was aspirated from the priming reservoir with the suction apparatus used clinically[3] (Fig. 10, *B*) and returned to the venous reservoir. Suction was provided by a Sigmamotor pump which was just totally occlusive and the flow rate was 1,300 c.c. per minute. Operative conditions were simulated by alternately sucking air and blood. In one experiment the plastic sponge in the cardiac suction reservoir was coated with Antifoam and in another experiment it was not.

*Made by the Mark Co., Randolph, Mass.
†A Lucite cylinder containing 6 Baxter blood filters in parallel.

Antifoam AF* (5 c.c.) was suspended in 300 c.c. of distilled water and mixed thoroughly. The plastic sponge was then immersed in the beaker and saturated. All excess droplets were shaken off the sponge and it was then wrapped and autoclaved.

Following this experiment the apparatus was broken down into 2 systems. The arterial system consisted of the arterial pump, the filter, the catheter, and the priming reservoir (Fig. 10, *C*). The recirculation system was composed of the recirculation pump, the oxygenator case, the screens, and the venous reservoir (Fig. 10, *F*). Using the same flow rate as before (3,000 c.c. per minute), 1,200 c.c. of the blood was circulated through the arterial system and the rate of hemolysis was determined. The filter was then removed from the system and the process was repeated (Fig. 10, *D*). Then the arterial catheter was removed and the blood was circulated through the pump and the priming reservoir (Fig. 10, *E*). In this way, we were able to estimate the effect of each component.

With the oxygen-carbon dioxide mixture still flowing, the recirculation pump was kept running at the same speed and 2,500 c.c. of the blood was circulated over the screens, into the venous reservoir, and back to the pump (Fig. 10, *F*). Next, the screens were removed

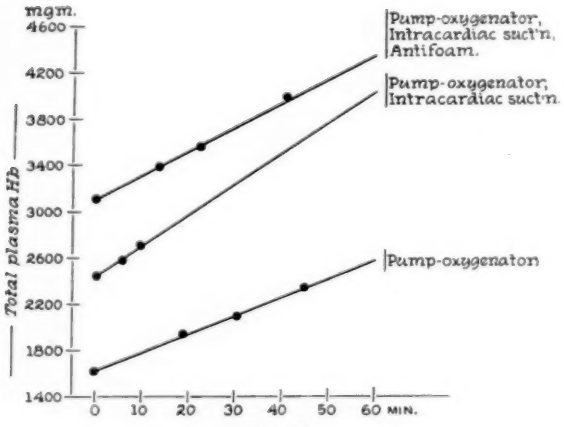

Fig. 11.

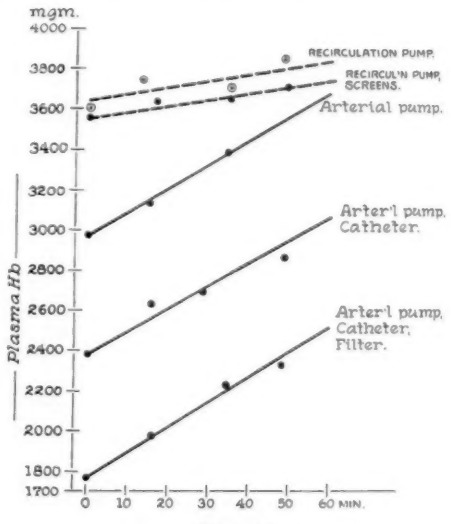

Fig. 12.

Figs. 11 and 12.—These represent the increase of plasma hemoglobin plotted against time of the various circuits listed.

and the blood was then pumped at the same rate into a Lucite reservoir set at the same height as the blood inlet to the oxygenator case. The blood then flowed by gravity into the bottom of the oxygenator case and was returned to the pump. This was done so that the nonocclusive pump would be working against the same gravity head in both experiments (Fig. 10, *G*).

TABLE I

PERFUSION CIRCUIT	MG. HEMOGLOBIN PER HOUR		
Entire pump-oxygenator system	960		
Entire pump-oxygenator system + suction	1,580	620	280
Entire pump-oxygenator system + suction + Antifoam	1,240		
Arterial pump + reservoir	700		
Arterial pump + reservoir + catheter	680		
Arterial pump + reservoir + catheter + filter	740		
Recirculation pump	185		
Recirculation pump + screens	180		

*Dow Corning Co., Midland, Mich.

Results.—In Table I it can be seen that the entire pump-oxygenator circuit produced 960 mg. of hemoglobin per hour (43 mg. per cent for the volume of blood used). When the intracardiac suction was used *with* Antifoam, the hemolysis increased to 1,240 mg. per hour. Suction *without* Antifoam, however, caused the rate of hemolysis to rise to 1,520 mg. per hour which was more than twice the amount caused by the suction apparatus with Antifoam. Further experiments have shown that the turbulence caused by air bubbling produced most of the hemolysis. The pump and suction tip contributed very little.

The arterial system alone produced 740 mg. per hour at a flow rate of 3,000 c.c. per minute. When the filter was removed, 680 mg. of hemoglobin per hour was released. When the catheter was also removed, the hemolysis was 700 mg. per hour. Thus the contribution of these pieces to the hemolysis was neglible as compared to that caused by the pump. Much has been said about the desirability of making connectors Venturi-shaped, of putting knife edges on them, and so forth to reduce blood damage.[2] There is no doubt that improper connectors may cause damage. The damage caused by the pump was far greater than that caused by our connectors, however. The connectors used in these experiments were of stainless steel and had uniform diameters. The edges were not ground down.

The recirculation system yielded 180 mg. of hemoglobin per hour when the blood was filmed on the screens. When the blood was circulated through the pump alone at the same flow rate, 185 mg. of hemoglobin per hour was released. These results suggest that the contribution of the screens and the gaseous atmosphere to the total hemolysis is insignificant compared to the pump.

DISCUSSION

In animal expirements, 3,700 to 8,000 mg. per cent of plasma hemoglobin has been required to cause renal damage.[6, 8, 10] The normal value of plasma hemoglobin in the human is 2 to 5 mg. per cent and this is removed by the reticuloendothelial system.[11] In hemolytic disease and incompatible transfusions, the plasma hemoglobin may rise rapidly to several hundred milligrams per cent and when the renal threshold of 135 mg. per cent is exceeded, hemoglobinuria will occur.[11] If the renal threshold is reached while hemolysis is produced by an extracorporeal system, 10 to 40 per cent of the plasma hemoglobin may be removed by the kidneys and the rest by phagocytosis and other mechanisms.[12] Measurement of the plasma hemoglobin in clinical cases is therefore not a measurement of the hemolysis being produced but is a measure of the balance between the rate of formation and the rate of elimination. Experiments were therefore done utilizing the extracorporeal system alone to exclude the effects of the body. In this manner, the true rate of hemolysis could be determined and the contribution of each component evaluated. This is possible because under fixed conditions the rate of hemolysis is constant, that is, the plasma hemoglobin increases directly with the length of time the blood is circulated for periods up to 6 hours.

The usual method of reporting plasma hemoglobin as mg. per cent is inherently misleading because corrections are not made for variations in the hematocrit and plasma volume. The same amount of free hemoglobin may be reported to vary considerably in different individuals unless dilution factors are taken into account. For an accurate comparison of erythrocyte damage, it is essential to calculate the actual amount of plasma hemoglobin being produced. Actual values will then be independent of the blood volume, and they can be readily obtained by multiplying the milligrams per cent of plasma hemoglobin by the volume of the plasma.

The importance of knowing exactly what the flow rate is during clinical perfusions can hardly be overemphasized. We believe these experiments demonstrate the necessity of using an occlusive pump if the determination of the flow rate is dependent upon preperfusion calibration. Moreover, we believe that the use of an occlusive pump in the manner described is the simplest and most reliable solution at present to the problem of insuring an accurate flow rate independent of resistance. It is evident that patients may be subjected to perfusion rates far lower than assumed if these principles are not understood and applied, and the lethal effects of inadequate perfusion rates may result. We wish to stress the importance of using rubber tubing in the pump, because we have not been able to use plastic tubing under conditions of total occlusion without splitting.

CONCLUSIONS

1. The use of a totally occlusive roller pump produced flow rates which were independent of the resistance, from 10 to more than 600 mm. Hg, and was shown to serve as a simple and reliable flowmeter. The rate of free plasma hemoglobin formation did not exceed safe limits, being 43 mg. per cent when 3,380 c.c. of blood was circulated at a flow rate of 3,000 c.c. per minute.

2. It was necessary to use rubber rather than plastic tubing in the pump, because plastic tubing split when used under conditions of total occlusion.

3. A totally occlusive DeBakey pump caused significantly less hemolysis than a totally occlusive Sigmamotor pump.

4. The major portion of hemolysis in the extracorporeal circuit was caused by the pump, and the contribution of the screens, connectors, filter, and other components was negligible. However, in the suction apparatus, bubbling phenomena were the major cause of hemolysis when not controlled.

5. The rate of hemolysis of heparinized blood was essentially the same as that of blood collected in A.C.D. solution.

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Discussion

DR. ROBERT S. SHAW (Boston, Mass.).—Hemolysis is rather more important than it would seem superficially, since it may be not a thing in itself alone, but just as an index of injury to the more subtle components of blood.

We would certainly agree that bubbles and frothing are a prominent source of hemolysis and so have looked a little harder at this in connection with the bubble-oxygenator. The first slide shows a percolator which we have pretended is a bubble-oxygenator. With this apparatus, you can vary the gasses you percolate with and the rate of gas flow.

The next slide shows some of the things that we found. Most of them were expected, like a linear correlation between rate of hemolysis and gas flow. This is done with split samples of old, second-hand bank blood. But if you look at the first and second columns, you see that you can accomplish almost a 50 per cent reduction in the rate of hemolysis in the bubbler by putting in wet, warm gas and avoid drying at the air interface, which is probably a prominent contributor to injury to blood at the air interface.

This is of great importance. Certainly, there are only a few people who bother to humidify oxygen in their oxygenators, but it may become of greater importance when we start to expand and push our duration of perfusion.

DR. McCAUGHAN (Closing).—Quite often you see the results of perfusions in which the plasma hemolysis of samples of blood taken from a patient after perfusion is shown. These values will be expressed in milligrams per cent and are important to that particular patient. Before one starts to use these values to compare the hemolytic effect between that patient and another patient or between that pump and another pump or other pump-oxygenator systems, he must realize that the blood that was put in the priming reservoir to prime the machine already is quite hemolyzed.

Also, the hemolysis caused by a low flow rate certainly will be different from that caused by a high flow rate. A hemolysis in milligrams per cent of 50 mg. per cent, for instance, in a large patient certainly is not the same thing as 50 mg. per cent in a small patient.

The hemolysis caused at the end of a 10-minute perfusion is certainly not the same amount of hemolysis that is caused at the end of 60 minutes of perfusion, even with the same concentration. While a patient is being perfused and free hemoglobin is being presented to the patient, some of this free hemoglobin is being removed simultaneously and some of it will be taken up by the reticuloendothelial cells. If the renal threshold of 135 mg. per cent is exceeded, some of it will also be removed in the urine and the results at the end of perfusion will not be actually the rate of hemolysis, but it will be the balance.

Different systems should be compared with these facts in mind.

THE RELATIONSHIP OF OXYGEN CONSUMPTION, PERFUSION RATE, AND TEMPERATURE TO THE ACIDOSIS ASSOCIATED WITH CARDIOPULMONARY CIRCULATORY BYPASS

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IN THE early postoperative period following intracardiac surgical procedures employing a variety of blood oxygenators, a shocklike state eventuating in death has been described. It has been suggested that this may be the result of severe uncompensated acidosis.[20] This may well be the case, especially in the presence of a severely damaged heart which is incapable of furnishing adequate circulation to the body, or this acidosis may reflect brain and cardiovascular damage sustained during the perfusion which renders the organism incapable of maintaining adequate homeostasis later.[4]

Embolization has been shown to result in death resembling in many respects that of normovolemic shock.[8, 10] Lack of adequate oxygenation will cause death after prolonged perfusion,[7] and oxygen intoxication has been invoked as another reason for mortality.[17, 24] But severe acidosis during the period of perfusion, particularly the "hypoxic acidosis" of low flow perfusions, is quite common.[1, 9]

Andersen and Senning[2] and Paneth and associates[23] have shown that low flows result in abnormally low oxygen consumption by animals during perfusions. This may amount to only 50 per cent of the control value. The latter authors observed in addition that the buffer base was reduced proportionately to the decrease in oxygen consumption. Under these circumstances normal arterial pH and pCO_2 are maintained by a severe reduction in the blood CO_2 content. These findings are similar to those observed in animals suffering from shock.[26]

It is the purpose of this paper to present evidence that with the use of an adequate oxygenator of the membrane type prolonged low flow perfusions of the whole body result in a considerably greater degree of acidosis postoperatively than do those perfusions conducted at rates approximating the normal cardiac output under anesthesia. This is reflected in the survival rate among those animals.

Postoperative chemical determinations show that the outstanding difference between survivors and nonsurvivors was the ability of the former to maintain the arterial CO_2 tensions within normal limits at all times. The animals

This work was supported in part by a grant from the Cleveland Area Heart Society.

Read at the Annual Meeting of the Society of University Surgeons, Boston, Mass., Feb. 13-15, 1958.

*Formerly Cleveland City Hospital.

which died were unable to do this and entered a vicious circle resulting in a profound state of acidosis, both metabolic and hypercapnic. It is also shown that hypothermia helps to prevent metabolic acidosis during perfusion. But this does not prevent the accumulation of lactic acid during the postoperative period unless active measures are taken to prevent tissue oxygen lack.

THE OXYGENATOR

The membrane oxygenator described in detail elsewhere[7] was used for these experiments. It is illustrated in Figs. 1 and 2, and the flow circuit is given in Fig. 3. In essence, the apparatus is designed to cause a very thin film of blood to flow between 2 plastic membranes on the outside of which oxygen is flowing. This permits diffusion of carbon dioxide from the blood and oxygen into it. The membranes are held by supporting plates with a grooved surface on one side and a screen on the other. Oxygen flows in the screens and grooves. Leakage of blood from the edges of the membranes is prevented by gaskets which surround each unit. Each pair of membranes presents 0.5 square meter of surface area to the blood flowing between them. Down the long side of each supporting plate is a ¼ inch groove. Blood enters through a distribution ring between the membranes at the corner and distends the membranes into the longitudinal groove along which it flows. As the pressure rises the membrane is distended into the ⅛ inch transverse grooves and allows the blood to flow across the short direction of the unit. Upon reaching the opposite side it is collected in a similar longitudinal channel to exit through the diagonally opposite corner from that through which it entered.

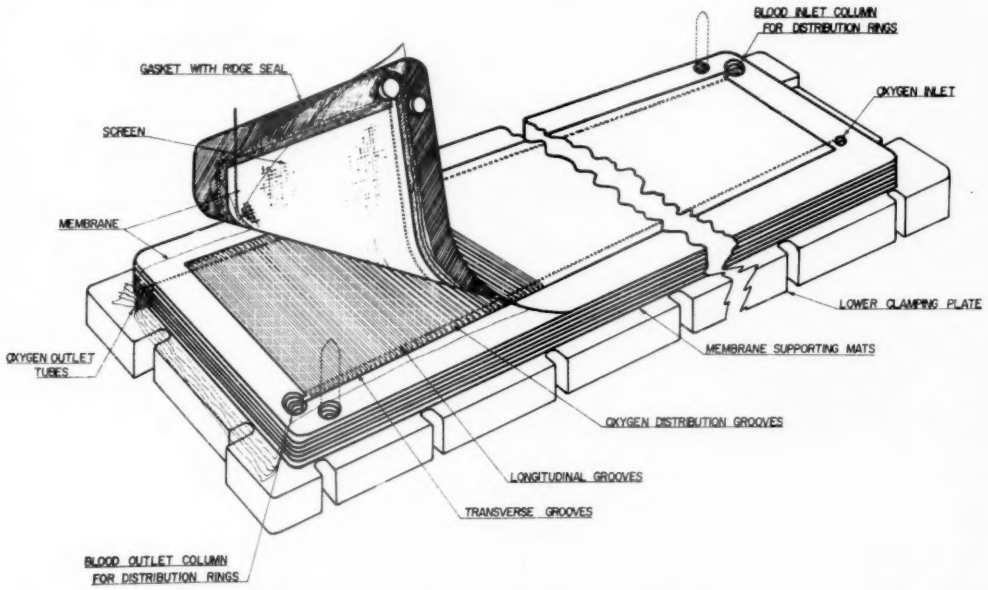

Fig. 1.—Diagram of membrane oxygenator.

Experiment has shown that with polyethylene 0.8 mil. in thickness approximately 3 c.c. of oxygen will be introduced into flowing blood by each unit in one minute. Using 0.75 mil. Teflon membrane, we increased this figure to 8 c.c. of oxygen per minute per unit. Thus, knowing the approximate oxygen consumption of a given animal or patient about to undergo perfusion, the right number of units may be set up to furnish the required oxygen delivery. These are stacked one on top of another. Blood is distributed to and removed from these multiple units by an internal manifold system. When the stack

of units is assembled, a heated metal rod is pushed down through the whole column of distribution rings perforating the plastic membranes as it goes. Thereby a blood channel is established to each pair of membranes.

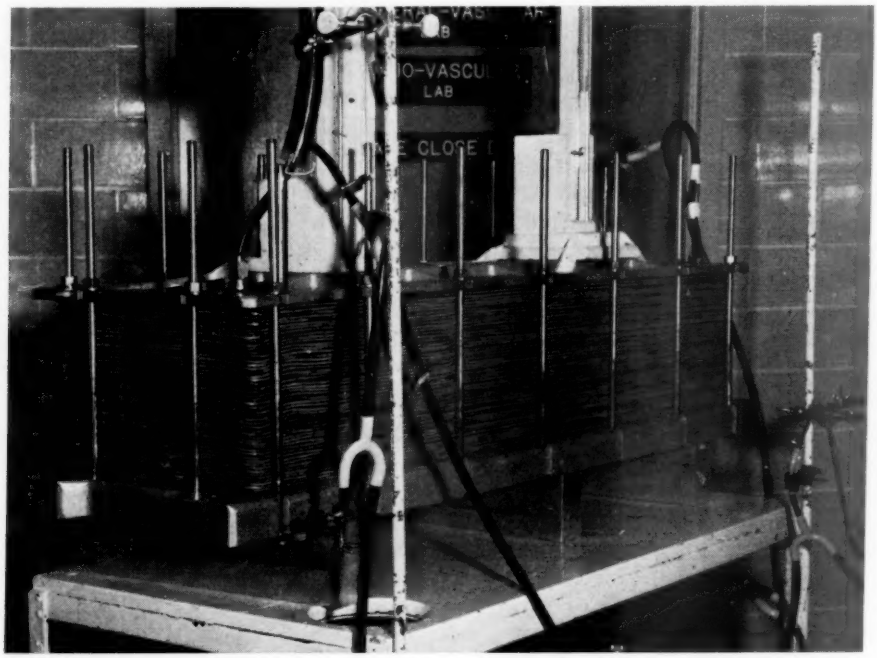

Fig. 2.—Photograph of membrane oxygenator.

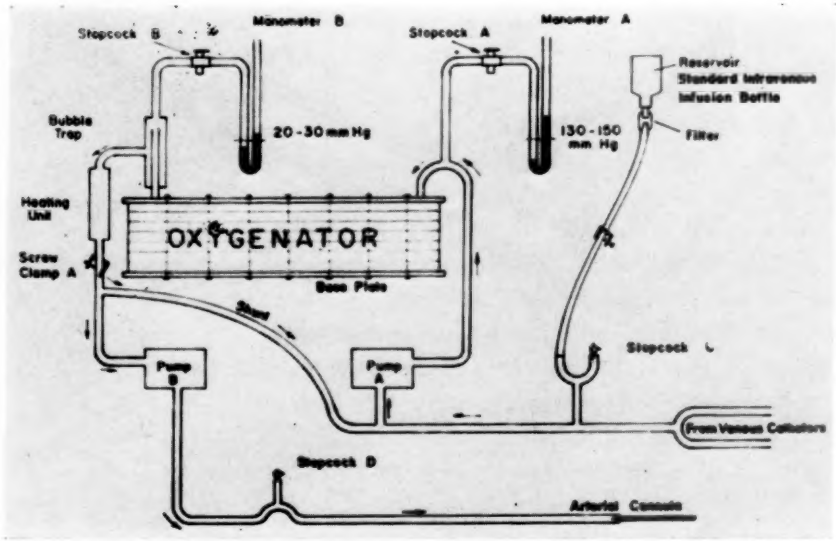

Fig. 3.—Membrane oxygenator tubing circuit.

As indicated in Fig. 3 a shunt is provided in the circuit. As long as pump *A* continues to deliver blood at a fixed rate and the outflow pressure of manometer *B* does not change, the volume of blood contained in the oxygenator remains constant in that the membranes are maintained on a given stretch. The apparatus is self-regulatory, for the volume of blood drawn through the venous catheters automatically equals that pumped into the arteries.

While in operation under full working pressure each unit contains approximately 50 c.c. of blood. This means that a 10-unit system including the tubing for a dog will hold approximately 700 c.c. of blood and be capable of supplying about 80 c.c. of oxygen per minute while, for an adult patient requiring 200 c.c. of oxygen per minute, a 30-unit system holding about 1,800 c.c. of blood has proved adequate. To date there is no evidence to suggest that fibrin emboli are created during its operation. Being a closed system it is free of the danger of entraining gaseous emboli in the flowing blood.

METHODS AND MATERIALS

Mongrel dogs weighing between 7 and 15 kilograms were employed for these experiments without special preparation. They were anesthetized with pentobarbital (27 mg. per kilogram of weight) administered intravenously. During the procedures blood pressure was recorded by a mercury manometer connected to the femoral artery. At the same site arterial blood samples were obtained. An electroencephalogram and an electrocardiogram were recorded on a Grass Model 3B oscillograph at intervals during each experiment.

Arterial and venous blood samples taken in oiled syringes were subjected to gas analysis by the method of Van Slyke and Neill.[29] By the Cambridge Glass Electrode, pH was determined. The lactic acid levels were obtained by the method of Barker and Summersen.[3] From the CO_2 content and the pH value, pCO_2 and buffer base were arrived at with the aid of the nomogram of Singer and Hastings.[28]

Oxygen consumption during the course of the perfusion was calculated by the formula: O_2 delivered (c.c./min.) $= \frac{\text{A-V Diff. (vol. \%)} - \text{flow rate (c.c./min.)}}{100}$. Blood for this analysis was drawn simultaneously from the inflow and outflow lines of the oxygenator during the period of total perfusion. Preoperative oxygen consumption under anesthesia was determined with a Collins Respirometer attached to a cuffed endotracheal tube.

PROCEDURE

Three groups of animals were perfused under normothermic conditions. Those in Group I ranged from 70 to 110 c.c. per kilogram per minute; Group II, 40 to 60 c.c. per kilogram per minute; and Group III, 26 to 36 c.c. per kilogram per minute. Similarly, 3 groups of animals were perfused at temperatures ranging from 28° to 31° C. Group IV had flow rates from 80 to 110 c.c. per kilogram per minute; Group V, 40 to 60 c.c. per kilogram per minute; and Group VI, 26 to 36 c.c. per kilogram per minute. To avoid the introduction of further anesthetic agents during the experimental procedure, donor blood was drawn from other dogs with local Novocain anesthesia into silicone-coated heparinized bottles* which contained 15 mg. of heparin per 500 c.c. of blood.

Through an intercostal incision in the third left intercostal space, a heavy ligature was placed loosely about the pulmonary artery. To insure adequate clotting in all oozing points, a period of one-half hour was then allowed to pass before the administration of heparin (3 mg. per kilogram of weight).

Plastic catheters of the largest possible diameter were introduced through the jugular and femoral veins into the venae cavae. A plastic cannula also of the largest possible diameter was introduced into the carotid artery and directed centrally. In instances when high flow rates were desired, 2 arterial cannulae and an extra venous catheter were employed in the opposite femoral vessels. These operations were conducted under clean but not aseptic conditions.

All parts of the oxygenator which touch blood were replaced for each perfusion with the exception of the blood distribution rings which were cleaned with acetic acid and scrubbed. Before connecting the apparatus to the animal, the wash saline within the oxygenator was replaced by a freshly drawn heparinized blood in sufficient quantity for full operating pressure at the desired flow rate. This prevented blood loss by the animal to the oxygenator.

*Kindly furnished by Abbott Laboratories, Inc., Chicago, Ill.

When blood pressure had become steady on partial bypass, total perfusion was instituted by occluding the pulmonary artery. This total perfusion was carried out for one hour in each instance.

Total perfusion was terminated by the removal of the ligature from around the pulmonary artery. Partial perfusion continued for a period of 3 to 5 minutes to make sure that the animal's heart was functioning normally. The arterial pump was shut off and an adequate volume of heparinized blood was transfused to the preperfusion level. Protamine dissolved in 50 c.c. of 5 per cent dextrose and water was administered by drip intravenously in amounts equal to the heparin given previously. Freshly drawn citrated blood was transfused thereafter slowly in amounts adequate to maintain a normal or near-normal blood pressure during the subsequent one hour.

When the perfusion was carried out under hypothermic conditions at approximately 30° C., the procedure was essentially the same as that described previously except that the water circulating in the heating unit on the outflow tract of the oxygenator was set at 26° to 28° C. during the initial period of partial perfusion. This ordinarily caused the esophageal temperature to fall to near 30° C. within 15 minutes after the onset of the perfusion. Thereafter the heating unit was kept at 30° C. When this hypothermic state had been obtained, total perfusion was started and continued for one hour. At the termination of the procedure rewarming was accomplished by immersion of the whole animal in warm water until a rectal temperature of 36° C. was attained. In the last 6 animals rewarming was accomplished by raising the circulating water in the blood heater to 46° C. This took as much as ½ to ¾ hour of extra partial perfusion to accomplish the desired level of rewarming. Thereafter both the normothermic and hypothermic animals were treated essentially in the same fashion.

Arterial blood samples were obtained before, during the perfusion, at the end of one hour, 3 hours, and in the majority of instances 6 hours after. If the animal survived, further samples were obtained in many instances at 18 and 24 hours later. The chest was closed after re-expansion of the lung. Prior to this, the chest cavity was examined for evidence of bleeding. If there appeared to be any marked degree of oozing, a chest tube was placed through an intercostal space and connected to water-seal drainage. This was left in place from 4 to 6 hours or until such time as the clotting mechanism had become fully re-established. No dogs are included in this series in which, for technical reasons, unreasonable degrees of hemorrhage took place within the chest during perfusion.

If animals did not survive, an autopsy was performed as soon after death as possible. In each instance care was taken to measure the amount of free blood present in the chest cavity. The remainder of the animals were sacrificed in 3 days.

RESULTS

Of the 43 animals subjected to total perfusion for one hour, all were alive at the end of the procedure. Twenty animals died postoperatively. One death occurred 4 hours postoperatively, one at 5 hours, and the majority between 6 and 12 hours. No deaths took place after 18 hours until sacrifice of the animals 2 to 3 days later. The mortality rates for the groups of animals perfused at high, medium, and low flow rates under normothermic and hypothermic conditions are given in Table I.

A. Normothermic Perfusions.—

Blood pressure: From an average mean arterial blood pressure of 120 mm. Hg after completion of the intrathoracic operative procedures, all animals showed a fall of pressure at the outset of perfusion. This was quite variable,

TABLE I. RELATIONSHIP OF MORTALITY TO PERFUSION RATE AND TEMPERATURE

	FLOW RATE (C.C./KG./MIN.) AND TEMPERATURE	NUMBER OF PERFUSIONS	NUMBER OF DEATHS	TIME OF DEATH (HOURS AFTER PERFUSION)	MORTALITY RATE (PER CENT)
Normothermic					
Group I	(70-110) 36-38° C.	14	3	7-18	21.4
Group II	(40-60) 36-38° C.	7	3	7-12	42.8
Group III	(26-36) 36-38° C.	8	5	4-10	62.5
Hypothermic					
Group IV	(80-110) 28-31° C.	9	4	6-16	44.4
Group V	(40-60) 28-31° C.	3	3	5-14	50.0
Group VI	(26-36) 28-31° C.	2	2	6-10	50.0

Group I perfused at rates between 70 and 110 c.c. per kilogram per minute had an average mean arterial blood pressure of 87 during the perfusions. This ranged from 70 to 105. The pressure tended to rise gradually during the period of perfusion. Those animals perfused at 40 to 60 c.c. per kilogram per minute had an average mean arterial pressure of 64 which ranged from 51 to 78. Low flow perfusions (26 to 36 c.c. per kilogram per minute) maintained an average mean arterial pressure of 52 mm. Hg during the perfusion. This ranged from 36 to 65 mm. Hg. During the course of these latter procedures the blood pressure gradually fell. Frequently near the end of the low flow perfusions it was necessary to add as much as 300 c.c. of blood to the system in order to maintain adequate venous pressure and perfusing conditions despite absence of external blood loss.

After the perfusions there was usually a prompt rise to near normotensive levels with the addition of transfused blood to restore normal venous pressure. Whereas the middle and high flow groups required seldom more than 200 c.c. of extra transfusion at the termination of the procedure, those in the low flow group were found to need regularly as much as 300 to 400 c.c. postoperatively. Restoration of the clotting mechanism made it difficult to follow the blood pressure for more than 2 hours. If, however, the blood pressure of a given animal had fallen below 60 mm. Hg by that time and it had not been corrected by the addition of further blood or the location of a bleeding point, such animals died within the next few hours. No particular blood pressure level during perfusion was found to be critical for survival afterward.

Blood loss in postoperative period: At autopsy of the 11 dogs which died, the volume of blood found free in the chest cavity, expressed in terms of the animal's preoperative weight, amounted to not more than 2 per cent. There were only 2 exceptions. One animal in Group I had bled a volume amounting to 3.6 per cent and another in Group III 3.1 per cent of their respective weights. This does not take into account the blood in the hematomas which formed at

times in the neck or groin wounds. The surviving animals which were examined at the time of sacrifice were discovered to have fluid and clots ranging from 0.96 to 2.13 per cent of their body weights.

Oxygen consumption: Of the 20 animals tested preoperatively under pentobarbital anesthesia, an average oxygen consumption of 7.6 c.c. per kilogram of weight per minute was found. This ranged from 6.3 to 10.1 c.c. per kilogram of weight per minute. As indicated in Table II, the animals in Group I, perfused at flow rates approximating that of the anesthetized dog, consumed an average of 6.4 c.c. per kilogram of weight per minute or 85 per cent of the average observed value in the control animals.

TABLE II. THE RELATIONSHIP OF OXYGEN CONSUMPTION TO PERFUSION RATE AND TEMPERATURE

	TEMPERATURE	FLOW RATE (C.C./KG./MIN.)	OXYGEN CONSUMPTION* (C.C./KG./MIN.) MEAN	RANGE	NUMBER	AVERAGE OF ANESTHETIZED CONTROL (PER CENT)
Normothermic	36°-38° C.	70-110	6.4	5.1-7.3	14	85
	36°-38° C.	40- 60	4.8	3.7-5.9	6	64
	36°-38° C.	26- 36	3.5	2.9-4.6	6	47
Hypothermic	28°-31° C.	80-110	5.1	4.4-5.7	9	68
	28°-31° C.	40- 60	3.5	2.7-4.1	6	47
	28°-31° C.	26- 36	2.6	2.0-3.6	4	35

*Determined from data obtained just before the end of a one hour total body profusion.

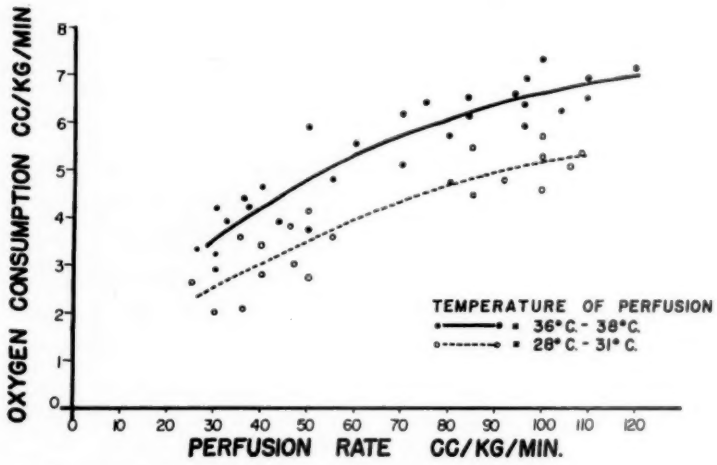

Fig. 4.—Relationship of oxygen consumption to perfusion rate.

At the other end of the scale this value showed further depression to 47 per cent in the animals perfused from 26 to 36 c.c. per kilogram per minute, which took place despite increased extraction of oxygen from what blood was circulating. Venous oxygen saturation commonly fell in Group III to values well below 20 per cent saturation although arterial blood was being delivered 93 to 95 per cent saturated. The relationship of oxygen consumption to perfusion rate is presented graphically in the solid upper curve in Fig. 4

in which are plotted data from each animal in which adequate information was available. It is apparent that the mortality rate following perfusions increased as the perfusion rate decreased from group to group, yet no relationship could be established between oxygen uptake and survival among the animals of any particular group.

Acidosis: In Fig. 5 are plotted the average arterial blood pH values for all animals of the high, middle, and low flow rate groups observed before, during, and after the period of perfusion. It will be noted that there was a depression of pH in all groups by the termination of the hour-long total perfusion. This was slight in the high flow group and became quite pronounced in the low flow group. Reference to Table III indicates that in these animals the arterial pH ranged at the end of perfusion from 6.97 to 7.20 in contrast to the animals perfused at high flow rates which ranged from pH 7.19 to 7.33.

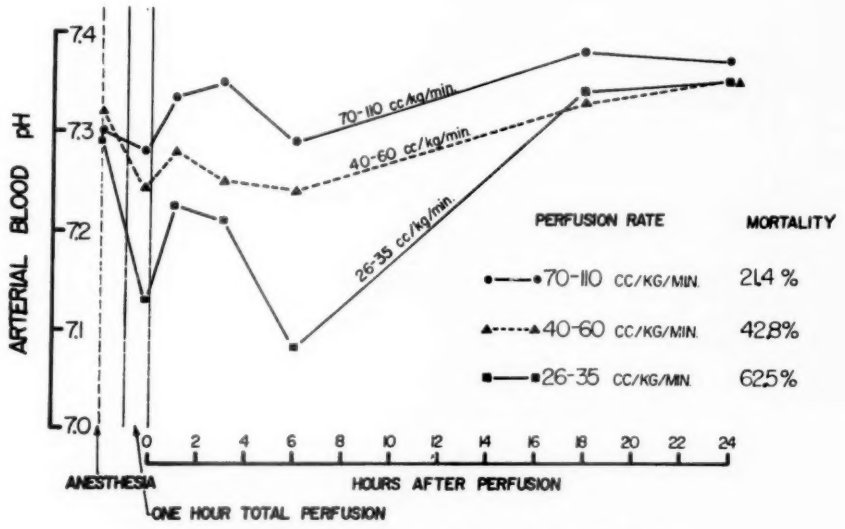

Fig. 5.—Relationship of acidosis to rate of perfusion.

In the postoperative period it will be noted that a transient rise of pH took place during the first hour after perfusion. During this period the dogs had been supported on a positive pressure respirator during the closure of the chest and blood pressure had been restored by the addition of extra transfused blood. Thereafter a fall took place in all 3 groups. This was most marked in Group III wherein the greatest mortality took place. The comparison made in Table III of the degree of acidosis observed in the survivors and nonsurvivors of each group during the critical first 6 hours of postperfusion shows that the nonsurvivors suffered by far the greater degree of acidosis. Fig. 6 presents the average pH values of all survivors and those of the animals which died in the normothermic groups. After the first 3 hours there was a clear demarcation in the range of pH, indicated by the vertical bars, found in those animals which were to survive and those which were to die.

TABLE III. THE RELATIONSHIP OF ACIDOSIS TO PERFUSION RATE AND TEMPERATURE
(Arterial Blood pH and pCO_2—mm. Hg)

FLOW RATE (C.C./KG./MIN.) TEMPERATURE	END OF PERFUSION						2 TO 3 HOURS POSTPERFUSION						4 TO 6 HOURS POSTPERFUSION					
	DEATHS			SURVIVORS			DEATHS			SURVIVORS			DEATHS			SURVIVORS		
	AVERAGE	RANGE	NUMBER	AVERAGE	RANGE	NUMBER	AVERAGE	RANGE	NUMBER	AVERAGE	RANGE	NUMBER	AVERAGE	RANGE	NUMBER	AVERAGE	RANGE	NUMBER
Normothermic																		
Group I																		
70-110	7.23	7.19-7.29	3	7.29	7.18-7.33	11	7.16	7.12-7.19	3	7.38	7.29-7.40	11	7.05	6.97-7.12	2	7.36	7.26-7.39	6
36°-38° C.	40	46-35	3	39	48-36	11	54	60-49	3	40	46-37	11	71	76-66	2	42	44-38	6
Group II																		
40-60	7.22	7.17-7.24	3	7.25	7.18-7.29	4	7.13	7.10-7.18	3	7.34	7.29-7.36	4	7.01	6.96-7.06	2	7.36	7.24-7.41	4
36°-38° C.	45	47-44	3	43	46-41	4	62	68-57	3	40	41-38	4	83	87-79	2	43	45-40	4
Group III																		
26-36	7.11	6.97-7.16	5	7.16	7.10-7.20	3	7.17	7.12-7.21	5	7.30	7.23-7.34	3	6.95	6.89-7.05	3	7.21	7.18-7.25	3
36°-38° C.	44	55-40	5	41	43-40	3	55	59-52	5	42	43-41	3	82	89-70	3	42	45-40	3
Hypothermic																		
Group IV																		
80-110	7.30	7.26-7.33	4	7.31	7.25-7.35	5	7.18	7.12-7.22	3	7.35	7.26-7.39	5	7.03	6.97-7.09	2	7.32	7.26-7.34	4
28°-31° C.							50	52-47	3	39	43-37	5	68	73-62	2	41	44-39	4
Group V																		
40-60	7.26	7.23-7.28	3	7.25	7.21-7.30	3	7.12	7.08-7.16	3	7.30	7.26-7.32	3	7.04	6.96-7.12	2	7.27	7.21-7.30	3
28°-31° C.							55	58-52	3	44	46-42	2	65	73-57	2	45	48-42	2
Group VI																		
26-36	7.19	7.16-7.22	2	7.20	7.16-7.24	2	7.13	7.09-7.17	2	7.26	7.20-7.31	2	7.01		1	7.32	7.27-7.38	2
28°-31° C.							60	62-57	2	46	50-41	2	74		1	42	46-37	2

Further examination of Table III shows that an earlier readjustment of pH took place in those animals which had been perfused at high flow rates than in those of Group III. However, by the following morning, 18 hours later, all survivors had returned to near normal pH levels.

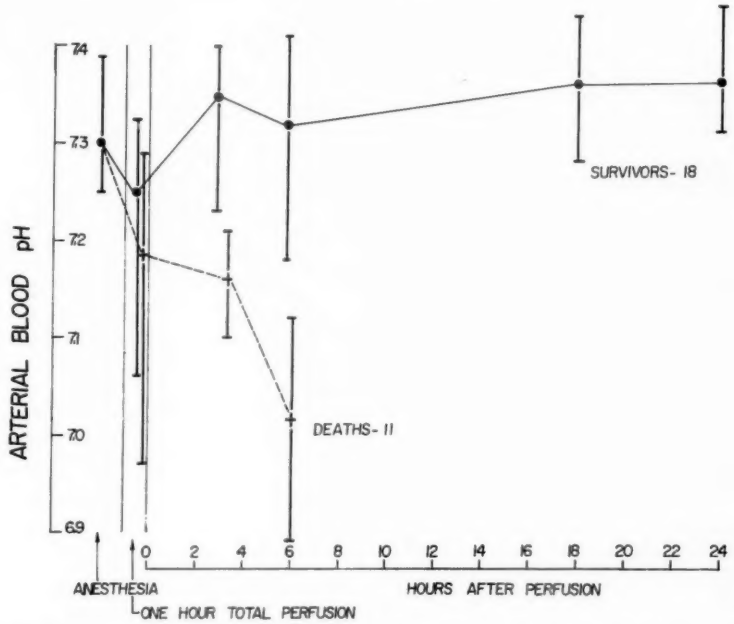

Fig. 6.—Comparison of degree of acidosis in survivors and nonsurvivors of normothermic perfusions (all groups).

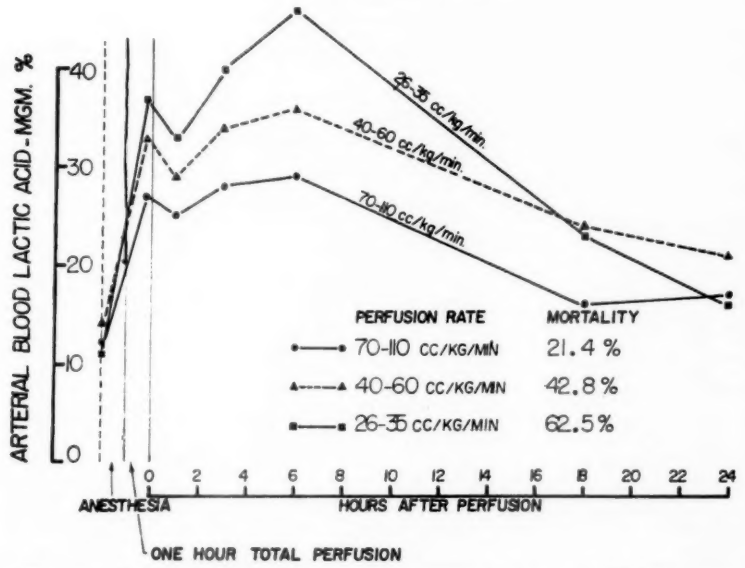

Fig. 7.—Relationship of lactic acid accumulation to perfusion rate.

Lactic acid accumulation: The blood lactic acid values are given in Table IV and the average values for each group are summarized in Fig. 7. By the end of one hour's total perfusion in the low flow group, lactic acid increased by an amount nearly twice that of the high flow group. There was a transient

TABLE IV. RELATIONSHIP OF PERFUSION RATE AND TEMPERATURE TO ACCUMULATION OF FIXED ACIDS AND REDUCTION IN BUFFER BASE
(Arterial Blood Lactic Acid—mg. %—and Buffer Base—mm./L.*)

FLOW RATE C.C./KG./MIN.) AND TEMPERATURE	END OF PERFUSION						2 TO 3 HOURS POSTPERFUSION						4 TO 6 HOURS POSTPERFUSION					
	DEATHS			SURVIVORS			DEATHS			SURVIVORS			DEATHS			SURVIVORS		
	AVERAGE	RANGE	NUMBER	AVERAGE	RANGE	NUMBER	AVERAGE	RANGE	NUMBER	AVERAGE	RANGE	NUMBER	AVERAGE	RANGE	NUMBER	AVERAGE	RANGE	NUMBER
Normothermic																		
Group I																		
70-110	28	23-33	3	27	21-30	11	36	30-38	3	26	21-34	11	42	34-50	2	25	22-30	6
36°-38° C.	36	35-37	3	37	35-38	11	34	34-35	3	42	39-43	11	33	30-35	2	41	38-42	6
Group II																		
40-60	31	28-33	3	34	24-38	4	42	36-47	3	28	24-31	4	46	41-51	2	30	22-35	4
36°-38° C.	36	34-38	3	36	34-38	4	35	34-37	3	39	38-40	4	33	30-36	2	41	39-45	4
Group III																		
26-36	38	31-42	5	35	30-39	3	46	42-53	5	28	23-32	3	58	45-70	3	34	31-39	3
36°-38° C.	30	25-33	5	31	30-33	3	33	30-36	5	38	36-40	3	29	28-31	3	36	34-38	3
Hypothermic																		
Group IV																		
80-110	22	18-28	4	24	17-33	5	38	30-43	3	26	20-31	5	48	39-57	2	25	21-32	4
28°-31° C.							34	33-36	3	39	38-41	5	31	29-33	2	39	37-40	4
Group V																		
40-60	28	21-32	3	25	20-31	3	44	35-50	3	29	26-33	3	49	41-55	2	30	26-34	2
28°-31° C.							32	31-34	3	39	38-40	3	31	30-32	2	39	37-40	3
Group VI																		
26-36	31	26-36	2	33	29-37	2	41	30-53	2	31	28-34	2	46		1	32	29-35	2
28°-31° C.							33	30-36	2	39	38-40	2	31		1	40	39-41	2

*Determined by nomogram of Singer and Hastings.

fall in lactic acid in each group following the period of perfusion with a continued rise thereafter. This attained large proportions as indicated in Fig. 7, particularly in the low flow group just prior to death of 5 of the 8 animals in this group. No significant difference was found in lactic acid accumulation between survivors and nonsurvivors by the end of perfusion, but a notable difference is present by 3 hours postoperatively. This is more marked in the period 6 hours after perfusion. It will be seen that the calculated values from buffer base are generally inversely proportional to the rise of lactic acid in the various groups.

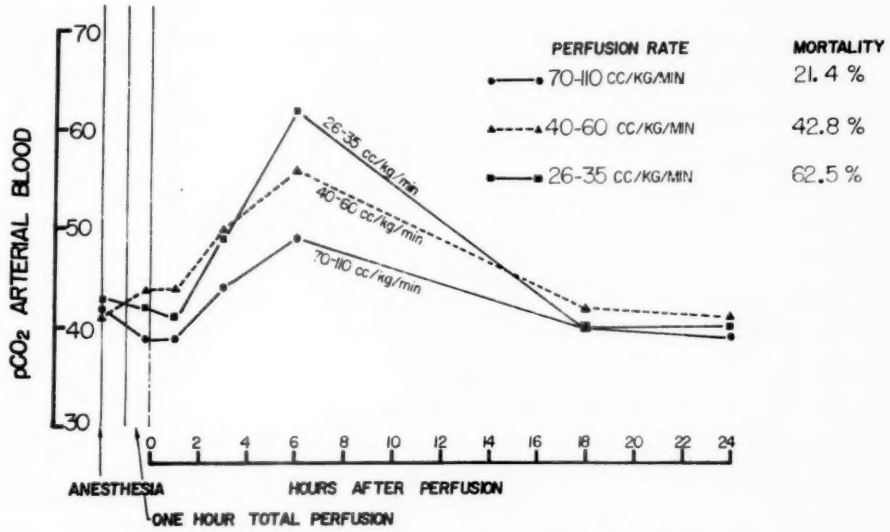

Fig. 8.—Relationship of arterial pCO_2 to perfusion rate.

Arterial pCO_2: The average values for the high, middle, and low flow rate groups are presented in Fig. 8. There was essentially no change from normal arterial pCO_2 values by the end of the period of total perfusion. This situation lasted until the end of the first hour postperfusion. Thereafter, there was a definite rise in all groups particularly noticeable in the animals perfused between 26 and 36 c.c. per kilogram per minute. The average value for all groups had returned to normal by 18 hours postoperatively, when only the survivors remained. Table III indicates no significant difference between the partial pressures of carbon dioxide observed in the arterial blood during the latter part of the one hour total perfusion between the survivors and the nonsurvivors. But at 3 hours a noticeable difference was apparent. The nonsurvivors without exception were outside the normal range, whereas all survivors had remained within the upper limits of normal. This was again true at 6 hours. The range of the nonsurvivors was at that time 66 to 89 mm. Hg in comparison with the survivors in which CO_2 tension ranged from 38 to 45 mm. Hg. This comparison is made in Fig. 9 which shows the precipitous rise of arterial pCO_2 in those animals of all normothermic perfusions destined not to survive.

Electroencephalogram: During the perfusions conducted with flow rates ranging from 70 to 110 c.c. per kilogram per minute there was little change noted during the course of the procedure in the electroencephalographic pattern

of the preoperative anesthetic state, unless, as occurred in several instances, the animals regained consciousness during the course of the perfusion. Postoperatively, consciousness returned within a short time to all of the animals of Group I. By contrast, in each instance there was a depression of the electroencephalographic pattern and potential during the course of the low flow perfusions. There was an increase in the number of alpha waves followed by slow waves of low potential and occasionally absence of potential in those animals in which the arterial pH fell to values below 7.0 during the perfusion. Those animals of Group III (perfused between 26 and 36 c.c. per kilogram per minute) which regained consciousness were slow to do so. The dogs of Group II perfused at 40 to 60 c.c. per kilogram per minute were variable in their response. In general, they showed less depression than the Group III animals, but were less alert postoperatively than the animals of Group I.

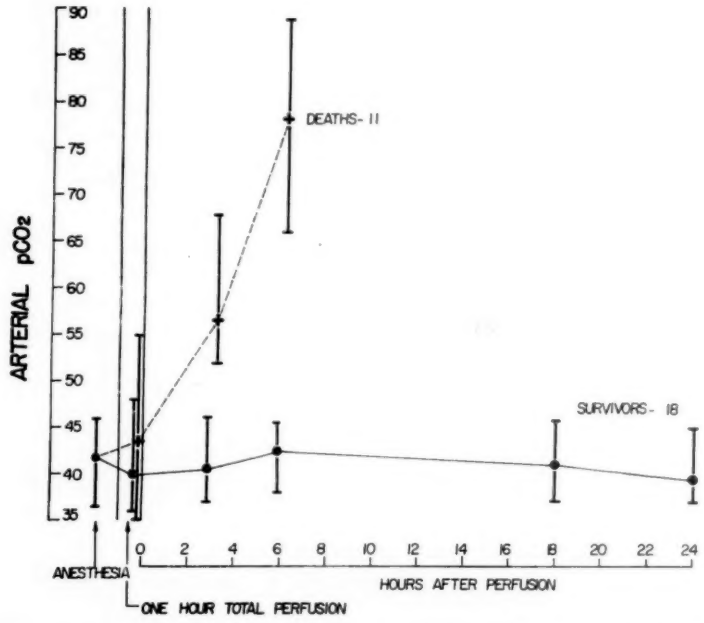

Fig. 9.—Comparison of the pCO_2 of survivors and nonsurvivors of normothermic perfusions (all groups).

B. Hypothermic Perfusions.—

Blood pressure and blood loss: The arterial blood pressure response to perfusion was essentially the same as in the normothermic animals with the exception that, once established, pressures did not tend to rise or fall during the procedure. Venous pressure did not fall in Group VI to the extent observed in Group III. Postoperatively the animals returned to near normotensive levels following rewarming and blood transfusion. Blood loss into the chest of the hypothermic groups which died amounted to an average of 2.1 per cent of the body weight. The greatest was 3.4 per cent.

Oxygen consumption: These animals at 30° C. on the average consumed about 75 per cent as much oxygen per minute as those perfused at comparable rates under normothermic conditions.

Acidosis: At a given perfusion rate less acidosis was observed among the hypothermic animals than the normothermic. The lowest arterial pH recorded in Group VI was 7.16 at the termination of the period of perfusion. No significant data are available for the period one hour after the perfusion in that rewarming was generally in progress at this time. However, at 3 hours it will be noted from Table III that the degree of acidosis among both survivors and nonsurvivors was essentially the same as that observed in the normothermic animals at the same time.

Lactic acid accumulation: The lactic acid increase during the period of perfusion was about two-thirds that observed in animals perfused under normothermic conditions. At 3 and 6 hours postoperatively this was no longer true; there was little difference in the amount of lactic acid present in the blood. This may well have been related to the shivering that took place in these dogs as they were rewarmed. There was a noteworthy difference in the lactic acid levels between the survivors and the nonsurvivors particularly at 6 hours. The former ranged from 39 to 57 mm. per cent whereas the latter showed values from 21 to 34 mm. per cent. A similar observation was made in regard to the arterial carbon dioxide tension. In the survivors this was within normal values in each instance, whereas in those animals which ultimately died the pCO_2 ranged from 62 to 74 mm. Hg.

Electroencephalogram: There was a moderate depression of the electrical potential of the brain waves in all animals undergoing hypothermic perfusions regardless of flow rate. This was particularly noticeable in the animals at lower flow rates, although complete absence of potential was not seen in any of these. All but 4 of the animals regained some degree of consciousness upon rewarming to about 34° C.

CLINICAL EXPERIENCE

In 10 consecutive clinical perfusions flow rates ranged in adults from 50 to 68 c.c. per kilogram per minute. For the one child in this series, the flow rate was approximately 85 c.c. per kilogram per minute. All patients awoke promptly with but one exception; an air embolism had occurred in this patient. She regained consciousness within 12 hours. In Table V are presented data from these 10 patients indicating that there was a moderate depression of pH from a control level of 7.39 to 7.33 during the course of the perfusion; the lowest pH observed during this period was 7.22. Three hours postoperatively the lowest pH observed was 7.26, with a mean value of 7.32 for all of the patients. There was a further slight depression to 7.29 on the following day with the lowest pH observed at this time being 7.24. By the second day, all surviving patients had returned to levels above 7.31. It is noteworthy that a normal arterial pCO_2 was maintained for all patients throughout the perfusion and the period after perfusion. Lactic acid accumulation took place, and at the end of perfusion the average level was approximately three times that of the control. This was reduced in the majority of the instances to normal levels by the third day postoperatively.

TABLE V. DEGREE OF ACIDOSIS AND pCO_2 ELEVATION IN TEN CONSECUTIVE CLINICAL PERFUSIONS

TIME	ARTERIAL pH		ARTERIAL pCO_2		LACTIC ACID	
	MEAN	S.E.	MEAN	S.E.	MEAN	S.E.
Preoperative	7.39	±.01	42.3	±1.4	10.8	±1.8
Anesthesia	7.38	±.02	35.0	±2.2	20.5	±4.3
End of perfusion	7.33	±.01	36.6	±3.1	29.3	±5.6
Postoperative						
3 hours	7.32	±.02	42.3	±1.5	26.6	±6.1
1 day	7.29	±.03	44.8	±2.1	28.3	±4.1
2 days	7.36	±.03	42.6	±1.6	17.1	±3.0

S.E.—Standard error.

DISCUSSION

The outstanding biochemical difference between the survivors and the animals which died following total perfusion for one hour was the postoperative development of profound uncompensated acidosis as shown in Fig. 6. It is suggested by the data in Table III and Fig. 7 that this was related to the inability of the nonsurvivors to maintain normal levels of carbon dioxide tension in the arterial blood as was done by those animals which survived.

This situation is not unlike that seen in the final stages of normovolemic or "irreversible shock,"[30] and it might be argued that the animals simply bled to death after the termination of the procedure. However, with only 2 exceptions the volume of blood found in the chests of those animals which died after normothermic perfusions amounted to less than 2 per cent of the body weight. This would hardly be sufficient to kill a normovolemic animal unless other factors were brought to bear which made him unable to resist this relatively small blood loss. Further, the quantity of fluid in the chests of the dogs sacrificed 2 days later was only slightly less.

In addition to blood loss other factors may contribute to the development of acidosis postoperatively: such as, heart failure, respiratory insufficiency, and brain damage which render the organism incapable of responding in normal fashion to maintain homeostatic conditions. It is true that certain of the animals were found to have some degree of atelectasis and congestion of the lung at autopsy, but this was not considered of sufficient extent to be lethal. Occlusion of the pulmonary artery for one hour may result in dilation of the right heart and a strain pattern in the ECG, but it seems peculiar that this would be responsible for death more often in the animals perfused at high flows than with low flows.

Embolization, oxygen lack, hypotension, and acidosis itself may result in brain damage sufficient to render the organism unable to maintain either normal respiration or circulation. Diesh and associates[10] observed a shocklike failure of the circulation in animals in which air embolization had taken place as demonstrated by specific gravity of tissues. Clowes and co-workers[8] observed that survival did not take place if the brain wave potential disappeared because of the introduction of fibrin emboli into the arterial circulation from an oxygenator then in use. It seems again unlikely that embolization should strike particularly at the animals perfused at low flow rates. The membrane oxygenator in

use for these experiments has been demonstrated to be capable of supporting circulation with survival for periods three times that employed in this group of experiments.[7] Although no filter is used in the circuit after the donor blood has been filtered, no evidence of fibrin embolus formation has been found.

Andersen and Senning,[2] using a filming oxygenator, and Paneth and associates,[23] with a bubble oxygenator, have demonstrated experimentally a decrease in the oxygen consumption of animals perfused at low flow rates to approximately 50 per cent of the control oxygen uptake under anesthesia. The data in Fig. 4 obtained with the membrane oxygenator confirm these findings. It should be pointed out that this resembles hemorrhagic shock, for Root[26] demonstrated that both in conscious and in anesthetized animals a depression of blood pressure to 50 mm. Hg resulted in a decrease of oxygen consumption to about one-half that of the control. As the flow rate of the pump-oxygenator was increased oxygen consumption by the animals increased also but never attained the normal value of 7.6 c.c. per kilogram per minute observed under barbiturate anesthesia in these experiments. Andersen showed in his experiments that oxygen consumption was related primarily to flow rate and not blood pressure. Although in Paneth's animals the oxygen consumption of brain and heart was the last to fall as flow rate was reduced, these organs began to use less oxygen as the flow fell below a level of approximately 40 c.c. per kilogram per minute. Using a polarograph implanted in the brain Kaplan[18] found a reduction in oxygen tension during periods of low blood flow. Bloor[4] observed that the oxygen tensions in the brains of animals in shock were essentially at normal levels until a critical blood loss was reached. Simeone, Husni, and Weidner[27] have recently demonstrated a fall of the oxygen tension in dog hearts which have been rendered hypotensive by bleeding. The part played by oxygen lack during perfusion in the ultimate failure of brain and heart in the dogs which died is suggested but impossible to assess fully at this time.

The development of "hypoxic acidosis" during perfusion is proportional to the flow rate as shown by the values given for lactic acid accumulation in Fig. 7. The accumulation of fixed acids in exercise,[14] shock,[26] and other situations in which an oxygen debt is developed is accompanied normally by hyperventilation and a reduction in arterial CO_2.[13] Such is the case with perfusions. DeWall[9] showed in 3 series of patients that the reduction of buffer base is inversely proportional to the flow rate. This, as indicated in Fig. 7, is the case with the dogs of these experiments. In a few instances very serious uncompensated acidosis took place during perfusion, especially in the low flow groups. This was accompanied in Group III by some depression of the brain wave potential. The significance of this is suggested by Clowes and associates[6] who showed that the brain wave pattern of anesthesia is further depressed by respiratory acidosis.

The consequences of this acidosis are suggested by the fact that animals perfused at low flow rates required considerably more blood added to maintain adequate venous pressure and perfusion conditions than those dogs which were perfused at rates approximating normal cardiac output under anesthesia.[11, 26]

Subsequently, these same animals required larger volumes of blood in the early postoperative period to restore venous and arterial pressure to near normal levels. This suggests a loss of vascular reactivity, as shown by Page[22] in shocked dogs, and perhaps a loss of blood by peripheral pooling.

Postoperatively all animals showed some fall in arterial pH. This has been true of our clinical experience and that of others.[1, 20] However, this was particularly marked in those animals perfused at low flow rates. Animals in which the arterial pH had fallen below 7.2 at 3 hours postoperative went on to death by further brain depression and inability to maintain the arterial carbon dioxide tensions within normal limits as shown in Fig. 9. Clinically the danger of severe acidosis regardless of cause in the postoperative period is well recognized and supportive measures such as blood replacement, the administration intravenously of sodium bicarbonate, and the use of artificial respiration have been recommended.[11, 21]

However, it appears that it is best to minimize oxygen lack and the development of "hypoxic acidosis" by the use of the higher flow rates especially if the perfusion is to be prolonged. Our clinical experience and that of Kirklin[19] appear to bear this out.

Another method suggested by Pierce[25] and Gollan[12] to avoid this acidosis with low flow rates is the concomitant use of moderate hypothermia. The data in Fig. 4 indicate that at 30° C. for a given flow oxygen consumption is reduced to about 75 per cent that of the normothermic animals. This is in general agreement with the results of Horvath and Spurr[15] in cooled intact animals. By the same token, as shown in Figs. 6 and 7, the acidosis and lactic acid accumulation of the perfused hypothermic animals was less at the end of the procedure than that in the animals perfused at normal body temperatures. Because of the crude rewarming techniques used in these experiments, the degree of acidosis later was as great as that of normothermic dogs, and the mortality figures are of questionable significance. Recently, Brown[5] has successfully used this technique for clinical operations employing a method of rapid warming and cooling.

SUMMARY

1. Three series of dogs were subjected to periods of one hour of total perfusion by the membrane oxygenator under normothermic conditions at rates of 70 to 110 c.c. per kilogram per minute (Group I), 40 to 60 c.c. per kilogram per minute (Group II), and 26 to 36 c.c. per kilogram per minute (Group III). They were followed for several days to observe the postoperative effects.

2. Oxygen consumption compared to control values ranged from 85 per cent in the high flow to 47 per cent in the low flow perfusions.

3. Acidosis and lactic acid accumulation during perfusion were inversely proportional to the perfusion rate despite the maintenance of normal CO_2 tensions.

4. Postoperatively acidosis increased in all animals but was marked in those animals which died. This was related to an inability of the latter to maintain normal carbon dioxide tensions in the blood.

5. The mortality rate of Group I was 21.4 per cent; Group II, 42.8 per cent; and Group III, 62.5 per cent.

6. During perfusions at 30° C. animals consumed about 75 per cent as much oxygen as the normothermic animals at a given rate of blood flow. Lactic acid accumulation and fall of pH were also less pronounced.

7. This development of postoperative acidosis is compared to clinical experience which suggests that high flow rates are desirable for any prolonged cardiopulmonary bypass.

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DISCUSSION

DR. BEN EISEMAN (Denver, Colo.).—About a year ago Dr. William Malette and I became curious about the fact that the venous return from dogs on a pump-oxygenator occasionally was inordinately bright red. Subsequently we found that this occurred when we had blown off too much carbon dioxide and thus had produced hypocapnea and a respiratory alkalosis in the animals. In the presence of such hypocapnea and alkalosis, oxygen is released slowly from hemoglobin so that even in the presence of fully saturated oxyhemoglobin so little oxygen is released to the tissues that tissue anoxia may result. This is the so-called Bohr effect, depending upon the dissociation curve of oxyhemoglobin, which you will remember is profoundly influenced by pH.

We have shown this effect actually to take place in a series of hyperventilated dogs in which cerebral tissue anoxia (as measured by increased brain tissue lactic acid concentrations) occurred despite near 100 per cent oxyhemoglobin saturation. These findings have practical implication for those using pump-oxygenators, for the paradox of highly saturated oxygenated blood may co-exist with tissue anoxia if there are hypocapnea and alkalosis.

Like the careful studies of Dr. Clowes, these data indicate the necessity of careful control of blood pH and CO_2 tension in a well-performed pump-oxygenator run.

DR. FRANCIS D. MOORE (Boston, Mass.).—As Dr. Clowes emphasized and implied, there are lots of things that produce low pH's and almost all forms of death are associated with agonal falls in pH. Shock, of course, of any type produces acidosis.

Our work has shown that venous oxygen saturation, lactates, and pH form a 3-dimensional linear relationship and that flow is the controlling factor—with low flow, low pH, high lactate and low venous oxygen saturation.

In addition, it is much easier to lower the pCO_2 on a pump than it is to oxygenate. Therefore, this point that Dr. Eiseman brought up is not as farfetched as one might expect. Some of the biochemical aspects of low pCO_2 (hyperventilation) coexist with the biochemical lesion of low flow and, ironically, they both produce lactic acidemia, but by different mechanisms, so that in that peculiar way, they are additive. If a pump with low pCO_2 is used on a patient who must later depend upon his own lungs with a fixed pCO_2 at 40, trouble is going to result and pH will show a late fall.

Some of the other changes in the first, second, and third postoperative day are slow enough to suggest alterations in renal function. We should not forget the kidneys when we are talking about acid-base balance.

DR. J. V. MALONEY, JR. (Los Angeles, Calif.).—About 10 years ago here in Boston, Drs. Beecher and Briggs, working at the Massachusetts General at the Overholt Clinic, were able to demonstrate degrees of metabolic and respiratory acidosis that produced pH's of 6.9 in patients undergoing routine thoracotomy. Dr. Derek and myself, working at the Brigham, had similar experiences. These levels of pH at metabolic and respiratory acidosis were said by the then current issues of *Best* and *Taylor* to be incompatible with life.

It is very interesting to hear that the patient undergoing cardiac bypass today is apparently, from the acid-base balance point of view, better off than the patient who in 1948 was simply undergoing anesthesia here in Boston.

DR. CLOWES (Closing).—I would point out that ordinarily, when anyone starts a perfusion, he generally has pretty alkalotic blood in the apparatus that has been circulating around getting ready to start. This invariably results in a fall in blood pressure in our experience and also it is known to cause a reduction in brain circulation. Either one of these things may produce a rise in lactic acid in its own right.

Dr. Moore has brought up the points of the relationship of shock to the problem. Essentially a low flow perfusion is a state of shock for as long as the perfusion goes on and the results are very similar. If carried to a long enough extent, you produce the state of normal volemic shock, sometimes called irreversible shock, that Dr. Wiggers and many others have described.

We considered the possibility that embolus formation to the brain, which we know can result in death, and acidosis and all these things may have played a part in the course of these experiments. However, it seems odd that it should strike the low flow group and not the high flow group. Usually, it is the other way around. The bubbles, if you are using an open type of oxygenator, are entrained at high flow or the fiber and collections are washed off at high flow.

Therefore, since we were able to carry on perfusions for as long as 3 hours with survival, we feel that embolus was not a part of these experiments.

OBSERVED CENTRAL NERVOUS SYSTEM RESPONSES DURING EXPERIMENTAL EMPLOYMENT OF VARIOUS PUMP-OXYGENATORS

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THE electroencephalograph has developed slowly as a research tool because the physiologic information delivered has been considered to be nonspecific and relatively insensitive. Recognizing these limitations, Gibbs and associates[4] in 1937, nevertheless, suggested the possibility of employing electroencephalographic techniques for controlling the depth of anesthesia. Faulconer,[3] Courtin,[2] and others further developed its application to surgical anesthesia and have shown that electroencephalographic monitoring is both desirable and practical.

With the development and clinical use of extracorporeal circulation with pump-oxygenators, the time-honored clinical landmarks of surgical and anesthetic tolerance such as blood pressure, pulse, respiration, and certain other objective criteria can no longer be entirely depended upon. Various electronic devices including the EEG therefore have come into use as adjuvants. Clowes and co-workers[1] in 1954 first reported using the EEG during cardiopulmonary bypass procedures. They indicated that a correlation seemed to exist between the severity of the EEG change during the period of bypass and survival. A very recent report by Theye and co-workers[10] describes the use of the EEG as a monitor in 100 patients during bypass operations when a filming-type oxygenator was employed.

During the past 4 years in our institution multiple observations have been made with the EEG during cardiopulmonary bypass in both laboratory and operating room using a variety of pump-oxygenator devices. It is the purpose of this report to summarize these observations and to assess the value of the EEG as an index of adequacy of pump-oxygenator performance.

METHODS AND PROCEDURE

Present-day heart-lung bypass techniques at Vanderbilt University Hospital are a product of studies made over a 4-year period during which microbubble, macrobubble, and

This investigation was supported in part by a grant from the John A. Hartford Fund, Inc., and Research Grant H-3348 from the National Heart Institute, U. S. Public Health Service.

Read at the Annual Meeting of the Society of University Surgeons, Boston, Mass., Feb. 13-15, 1958.

*National Research Council Fellow in Medical Sciences.

filming oxygenators, in the order listed, were used. During this period the electroencephalograph has been used to monitor approximately 150 dogs and 23 patients. Unipolar and bipolar EEG tracings were recorded on a 4-channel Offner machine using needle electrodes embedded in the scalp overlying the motor cortex bilaterally. The left ear was used as an indifferent point of reference for unipolar records. A simultaneous electrocardiogram (Lead II) was also recorded. After obtaining base line tracings, continuous records were made during and immediately following the use of the pump-oxygenator.

Microbubble Oxygenator.—The EEG was monitored in 100 mongrel dogs and in 3 patients during cardiopulmonary bypass with a Gollan microbubble pump-oxygenator. Sodium pentobarbital administered intravenously at a constant drip was the anesthetic agent generally employed in animal experiments. Respirations were maintained by a demand-type respirator except during the bypass procedure at which point the lungs were allowed to collapse. Standard techniques of cannulation, heparinization, and monitoring of arterial and venous pressures were used. An intermittent suction and pressure pump circulated the blood and was controlled by a simple electronic circuit containing 2 electromagnetic relays and energized by the perfusate coming in contact with positive and negative electrodes.[5] Hypothermia (26° to 28° C.) was employed in some of these animals. At normal temperatures flow rates of about 30 c.c. per kilogram per minute were striven for while the rates of flow during lowered body temperatures were somewhat lower.

Macrobubble Oxygenator.—In 25 dogs and 7 patients the EEG was monitored during bypass procedures with the Lillehei-DeWall type of oxygenating device.[7] Gravity drainage and a Sigmamotor pump were used in circulating the blood and flow rates of 30 to 50 c.c. per kilogram per minute were commonly obtained. Ether was used exclusively for anesthesia. Standard techniques were otherwise rigidly followed.

Filming Oxygenator.—In 25 dogs and 13 patients the EEG has been monitored during bypass procedures using a modified Gibbon type of apparatus. Gravity drainage and DeBakey type pumps were used.[6] Flow rates in the range of 70 to 120 c.c. per kilogram per minute (2 to 2.4 L. per square meter per minute) were employed. Light ether anesthesia was used routinely. Ether vapor was administered directly into the oxygenating chamber as required when the pump device was in operation. Standard techniques of cannulation, heparinization, and monitoring of arterial and venous pressures and venous oxygen saturation were utilized.

RESULTS

Microbubble Oxygenator.—Fig. 1 illustrates the usual electroencephalographic alterations encountered in dogs when the microbubble oxygenating device was used without hypothermia during cardiopulmonary bypass procedures. Within one minute marked alterations appeared although flattening of the waves to an isoelectric level was not apparent until about 15 minutes of pumping had elapsed. Previous work in this laboratory had demonstrated that azygos flows in the range of 12 c.c. per kilogram per minute were sufficient to maintain cortical electric activity for approximately 30 minutes.[9] Despite flows of more than twice this with the microbubble oxygenator, these electroencephalographic results occurred consistently. Only 20 per cent of these animals survived and many of the survivors demonstrated serious neurologic disorders. Death often occurred from one to 24 hours after the procedure. Routine microscopic studies conducted on brains from these animals failed to reveal histologic changes which could be implicated as the underlying cause of death.

In these experiments with the microbubble oxygenator it became clear that the longer the duration of electroencephalographic flattening, the less

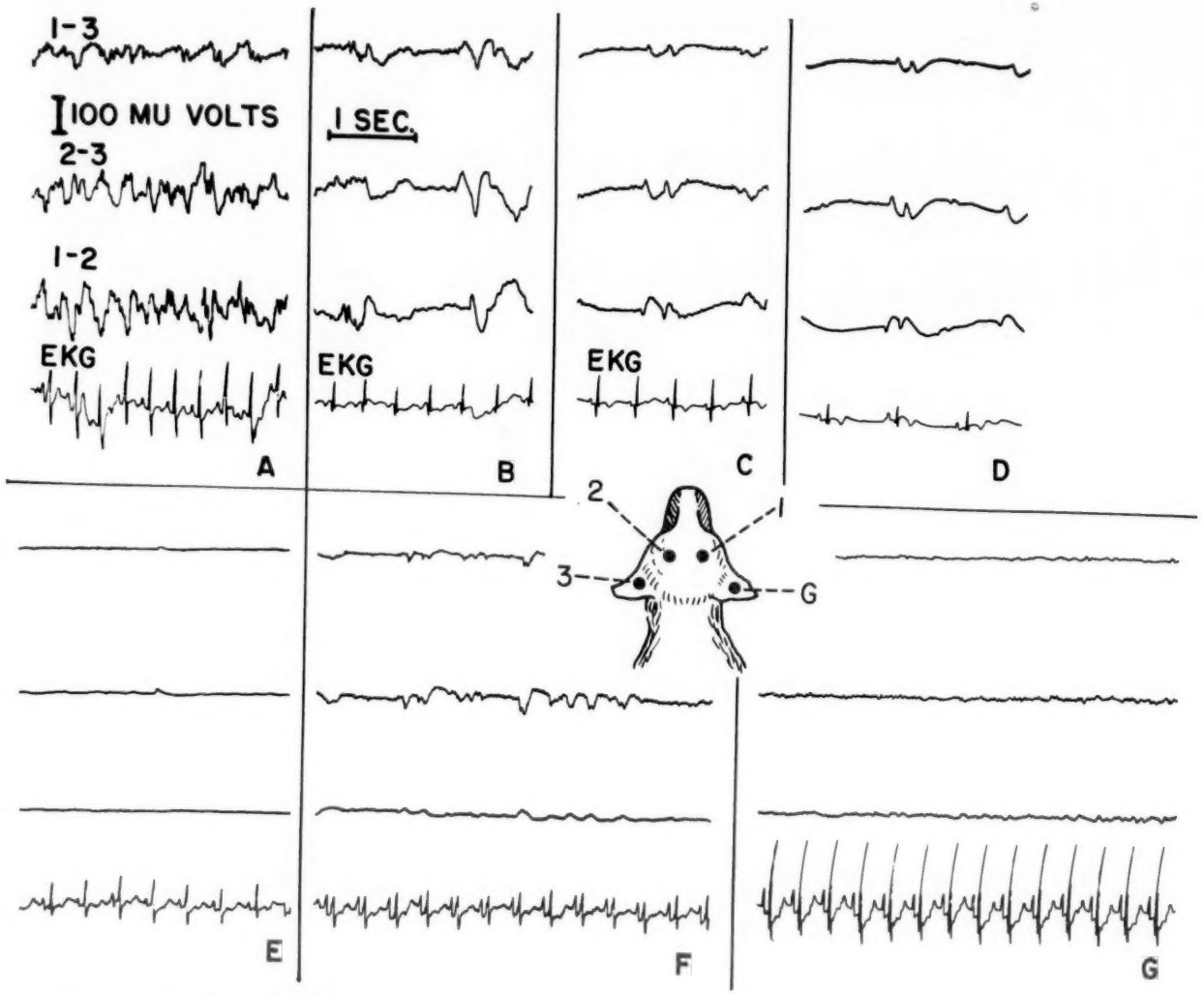

Fig. 1.—Dog No. 683. EEG record samples obtained during cardiopulmonary bypass using a microbubble oxygenating device. *A*, Prepumping tracing, intravenous Nembutal anesthesia. *B*, 1 minute on pump. *C*, 6 minutes on pump. *D*, 15 minutes on pump. Negative deflections are artifacts produced by the electronic control circuit used with this pump. *E*, Immediately following resumption of cardiac circulation. *F*, 1 hour postpump. *G*, 3 hours postpump. The record demonstrates very marked alterations.

likely the chance of survival. It was a general impression that some improvement in the electroencephalographic activity might have resulted if flows could have been increased. When hypothermia in the range of 28° to 30° C. was used with the microbubble oxygenator similar electroencephalographic responses were observed although survival was somewhat more likely. Another group of experiments reported elsewhere demonstrated this.[9]

Macrobubble Oxygenator.—When the macrobubble oxygenator was employed whether experimentally or clinically, certain characteristic electroencephalographic responses soon developed with great consistency. Almost immediately after the actual bypass was instituted, considerable slowing and decrease in amplitude were noted (Fig. 2, *F*). Improvement of the electroencephalographic record occurred within 2 to 3 minutes with a return to a close approximation of the prepump pattern (Fig. 2, *G*). This level of electrical activity, however, did not persist. At 20 to 30 minutes of pumping the appearance of slow waves and a decrease in amplitude was characteristic (Fig. 2, *H*). This level of electroencephalographic activity appeared gradually. Isoelectric changes were never encountered (except in emergency situations when circulation became grossly inadequate) even when the bypass procedure was prolonged to 45 or 60 minutes. When normal cardiac activity was immediately resumed following the pumping procedure and satisfactory arterial pressure was maintained, the electroencephalographic pattern within 1 to 2 minutes approached the base line prepump appearance.

During early experience with clinical application of the macrobubble oxygenator, 25 to 50 mg. of sodium Pentothal was occasionally added to the perfusing blood in order to maintain satisfactory anesthesia. The addition of this agent was soon discarded, however, because a prolonged period of unconsciousness was common and was attributed to this addition of a central nervous system depressant. It was noted subsequently that when ether vapor was bubbled through the oxygenating system recovery of consciousness again was prolonged. It became obvious that during the pumping procedure (especially if it were prolonged over 20 minutes) and afterward only small quantities of these anesthetic agents could be given lest the EEG activity be reduced to a minimum. The "trauma" produced by the macrobubble type of extracorporeal circulation alone was apparently sufficient to effect a degree of cerebral depression. Mortality figures were again excessively high in both experimental and clinical applications. Deaths during the first 12 to 24 hours after bypass with failure to regain consciousness were common. Causes of such deaths could not be determined by routine measures. This type of macrobubble oxygenator proved to be unsatisfactory in our hands, although it was certainly a great improvement over the microbubble device as measured by electroencephalographic standards.

Fig. 3 is again a representative electroencephalographic record during cardiopulmonary bypass with macrobubble oxygenation. Sections *C* and *D* at 10 and 15 minutes, respectively, graphically illustrate the change from the base line pattern. The high amplitude, fast activity seen in *E* and *G* remain

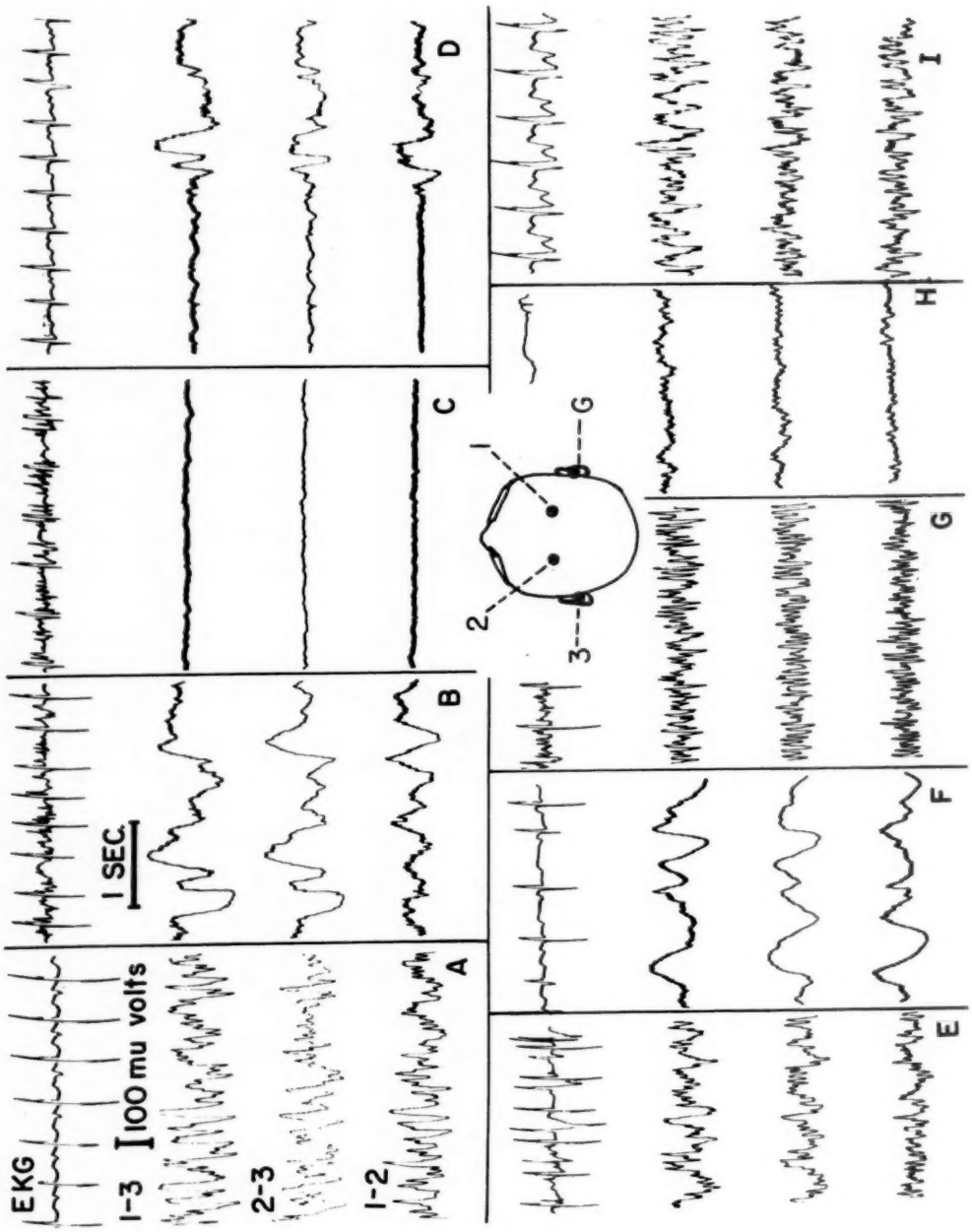

Fig. 2.—EEG record of Patient S. M. during cardiopulmonary bypass using a macrobubble oxygenator. *A*, Prepump tracing, light ether anesthesia. *B*, Defective tracing occurring during venae cavae cannulation with severe drop in blood pressure (50/40). *C*, 50 seconds following first appearance of EEG change. *D*, 30 seconds after restoration of normal blood pressure (120/80). *E*, Prepump tracing following cannulation. *F*, Appearance after 20 seconds on pump. Blood poorly oxygenated initially. *G*, 5 minutes on pump. Wave activity typical of light Pentothal anesthesia. *H*, 20 minutes on pump. *I*, 5 minutes after restoration of normal circulation.

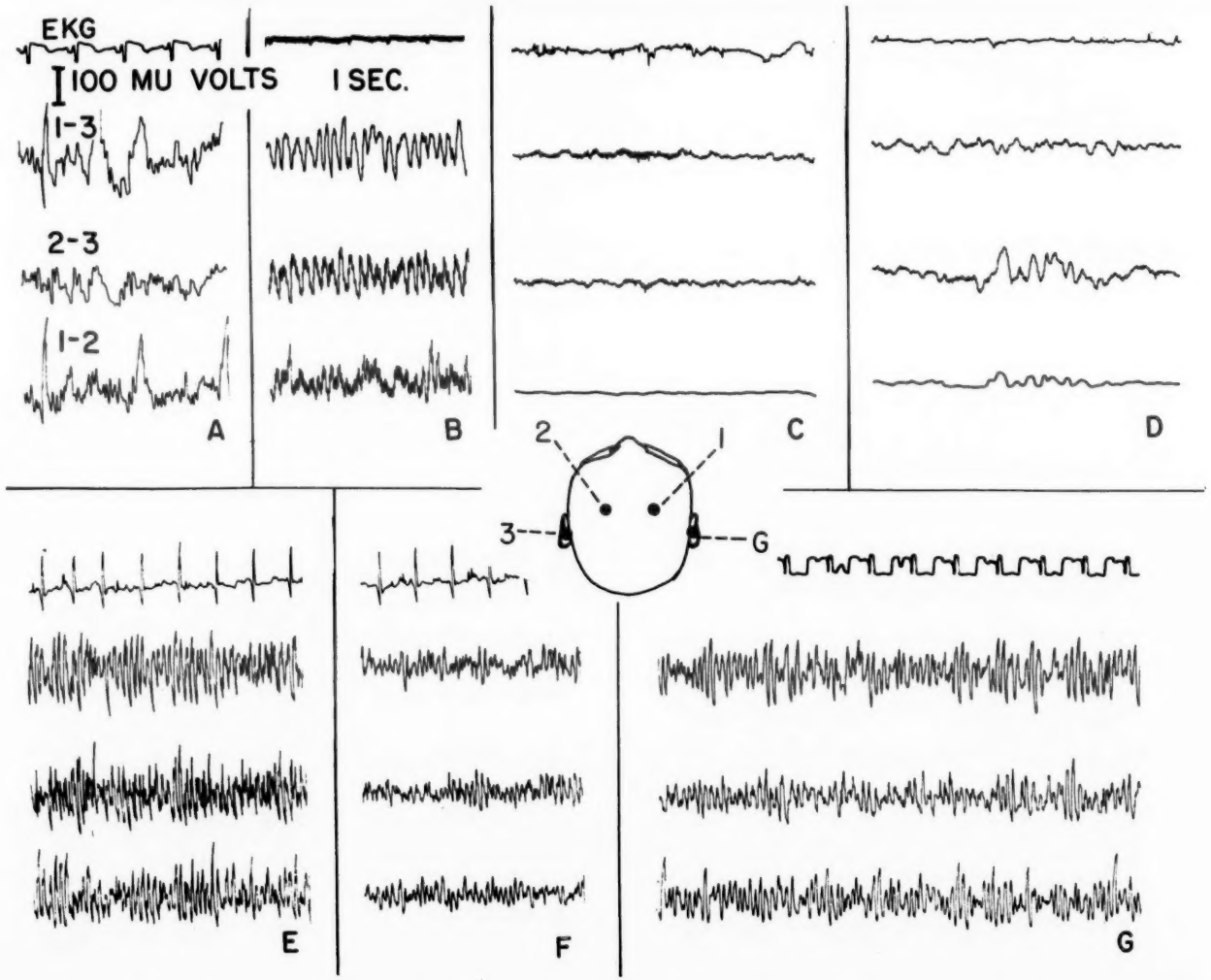

Fig. 3.—EEG record of Patient G. D. during bypass with a macrobubble oxygenator. *A*, Prepump appearance, light ether anesthesia. *B*, 2 minutes of pumping. *C*, 10 minutes. *D*, 15 minutes on pump. *E*, 30 minutes following pumping procedure. See text for detailed information on samples *E*, *F*, and *G*.

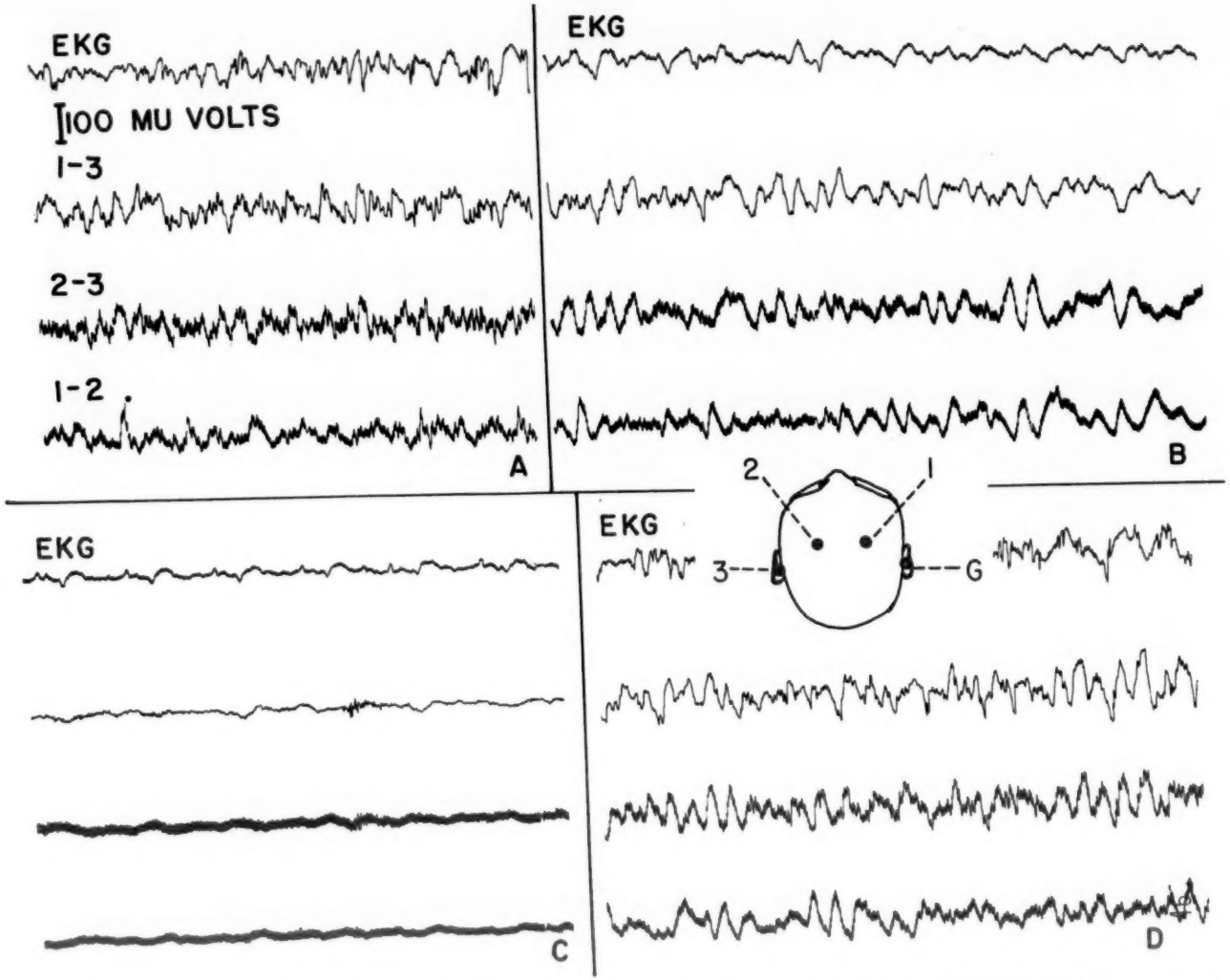

Fig. 4.—Patient S. M. (same patient represented in Fig. 2). One hour following procedure cardiac arrest occurred. Note EKG. *A*, Cardiac massage in progress. *B*, 10 seconds later, massager's hand is tiring. *C*, During changing of hands there was prompt disappearance of EEG activity. *D*, Immediate resumption of massage. Restored EEG activity is quite good.

unexplained. Carbon dioxide combining power measured at the time *E* was recorded was 7.8 mEq. per liter and the arterial pH was 7.2. Sodium bicarbonate was administered intravenously with a response in the pattern as illustrated by *F*. The clinical findings were indicative of deep anesthesia. This same high amplitude, fast activity electroencephalographic response was seen in several other patients. It may be the result of profound metabolic acidosis.

Fig. 4 is included because it illustrates the sensitivity of the electroencephalogram in one of the complications encountered in cardiac surgery. Patient S. M. (initial tracing illustrated in Fig. 2) developed ventricular fibrillation about an hour following the pumping procedure and cardiac massage, electrical defibrillation, and so forth were immediately instituted. Fig. 4, *A* shows the record as vigorous massage was in progress. *B* is a short time later as the massager's hand was tiring, and *C* was taken during the changing of hands. *D* is immediately following this shift with the resumption of more effective massage. It should be pointed out that such an instantaneous electroencephalographic response is more likely following a period during which the reserve tissue metabolites and oxygen have been depleted. In this instance a period of hypotension had preceded the cardiac arrest.

Filming Oxygenator.—A remarkable degree of electroencephalographic stability was maintained in all subjects during cardiopulmonary bypass with the filming oxygenator at high flows in both experimental and clinical application. There was no detectable abnormality of the electroencephalographic response, clinically or experimentally, immediately following the substitution of the extracorporeal circuit as was characteristically observed with bubble oxygenators. The fluctuations in the record during the usual bypass procedures, some of which were carried out for 60 to 90 minutes, were apparently directly related to the depth of anesthesia.

Fig. 5, *B* and *C* illustrates the usual electroencephalographic response in dogs at the end of 1 and 2 hours of cardiopulmonary bypass with the filming oxygenator. No obvious change in rate or amplitude is apparent. In this particular animal, the procedure was prolonged to a total of 5 hours and Fig. 5, *D* demonstrates the pattern at this point.

Fig. 6 illustrates the EEG response of a patient during 65 minutes of heart-lung bypass with the filming apparatus. The degree of change, if any exists, is certainly insignificant.

Fig. 7 demonstrates the characteristic effects on the electroencephalogram when ether vapor is introduced into the filming oxygenator during a bypass procedure. Twenty-five minutes of pumping had elapsed and with the patient very "light" (Fig. 7, *B*) ether vapor (one-fourth ounce in this instance) was again administered. Within 20 seconds (*D*) the record demonstrated marked slowing and at 30 seconds (*E*) deep anesthesia was indicated. The EEG tracing can thus indicate the need for added anesthesia during extracorporeal circulation and can also promptly reflect excessive use of the agent employed. Anesthetic requirements are reduced by prolonged pumping (over 45 to 60 minutes),

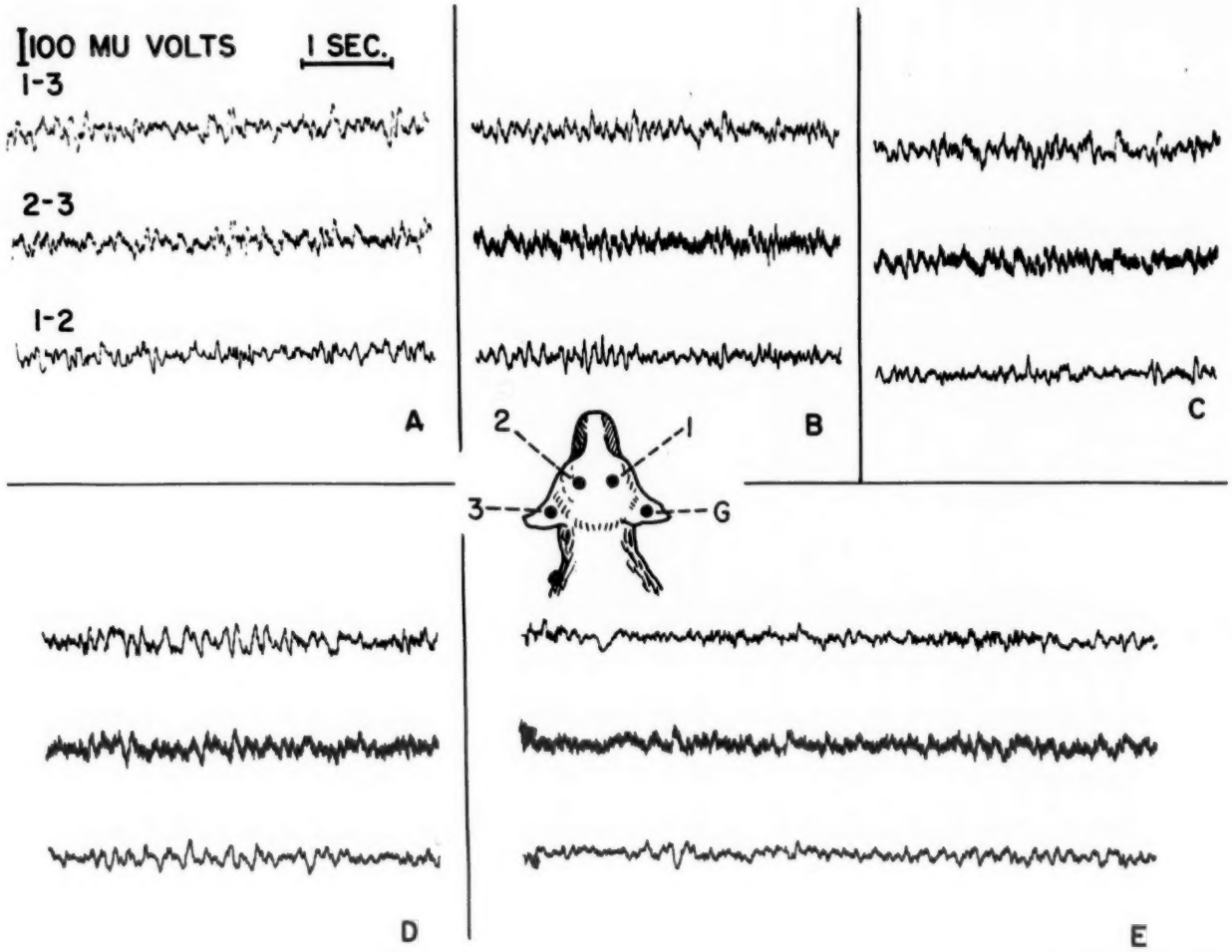

Fig. 5.—EEG record of dog during 5-hour bypass procedure using a filming oxygenator. *A*, Prepump tracing, light ether anesthesia. *B*, 1 hour. *C*, 2 hours. *D*, 4 hours. *E*, 5 hours on pump. There is some slowing with a definite decrease in amplitude at this point.

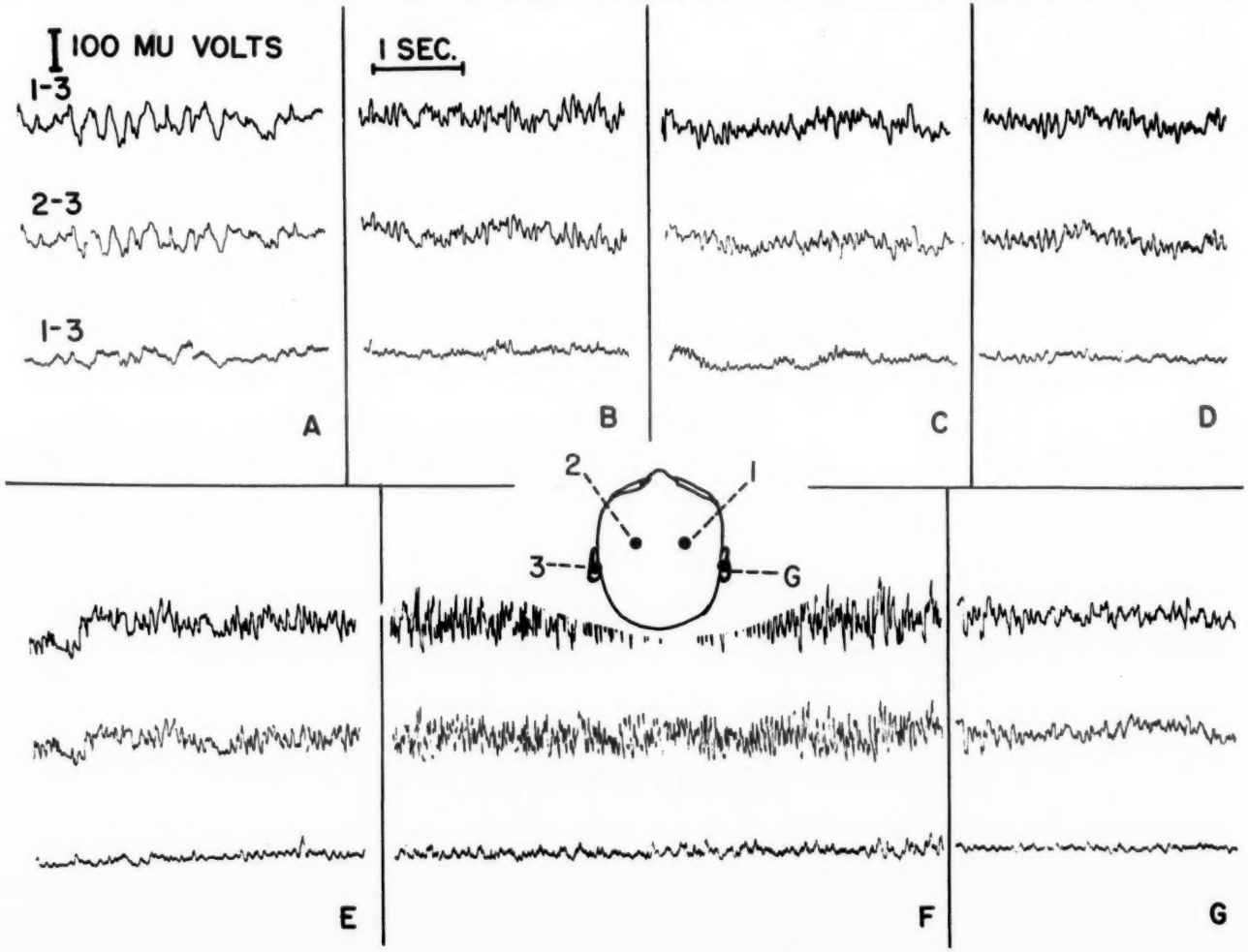

Fig. 6.—Patient B. C. EEG record during bypass procedure using a filming oxygenator. *A*, Prepump tracing, ether anesthesia (deep). *B*, 10 minutes on pump. *C*, 25 minutes on pump. *D*, 65 minutes on pump. *E*, 2 minutes after resumption of normal cardiac circulation. *F*, 5 minutes off pump. Pupils dilated and fixed. High amplitude, fast activity may be related to severe acidosis. *G*, 30 minutes after pumping procedure terminated. Normal (ether anesthesia) activity.

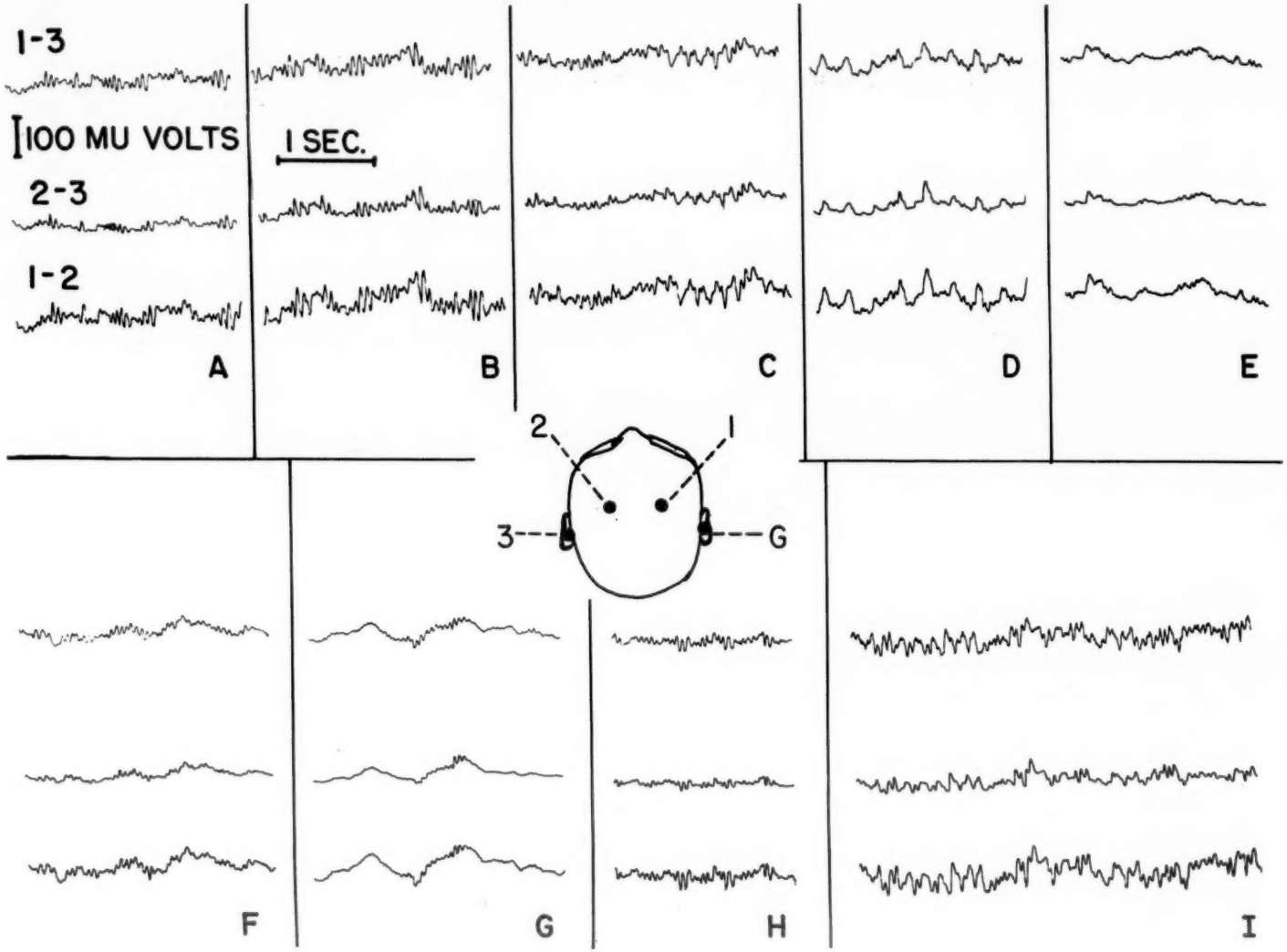

Fig. 7.—EEG tracing of Patient J. S. during cardiopulmonary bypass using a filming oxygenator. *A*, Prepump tracing. *B*, Tracing after 25 minutes on pump. Record indicates light plane of anesthesia. *C*, Ether vapor introduced at this point. *D*, 20 seconds after administration of one-fourth ounce of ether. *E*, 30 seconds afterward. Record demonstrates excessive depth of anesthesia. *F*, Appearance of EEG 2 minutes after restoration of cardiac circulation. Blood pressure dropping (100 to 70 mm. Hg). *G*, Pressure 40 at this point. Bypass again instituted. *H*, 20 seconds after re-employment of pump-oxygenator. Excellent restoration of EEG activity resulted. *I*, Record at end of operation.

drops in blood pressure and/or flow rates, and the quantity of anesthesia previously administered. The summation effect of these various factors is clearly indicated by the cortical electrical activity and allows closer anesthetic control.

Violent respiratory movements which were often encountered during the bypass procedures with micro- and macrobubble oxygenators were initially thought to be related to medullary oxygen insufficiency. These supposed manifestations of "air hunger" persisted, however, even after employment of the filming oxygenator which was associated routinely with stable electroencephalographic activity and good survival results. With good evidence of the adequacy of cerebral blood flow and oxygenation, it was then appreciated that this respiratory activity during cardiopulmonary bypass was an exaggerated Hering-Breuer reflex, and by merely inflating the lungs excessive respiratory movements could be eliminated.

COMMENT

This study has demonstrated that the EEG is a reliable and sensitive indicator of the adequacy of cerebral perfusion by oxygenated blood during bypass procedures with pump-oxygenators. Observations during the use of microbubble and macrobubble oxygenators have consistently shown undesireable alterations in the EEG which apparently reflect suboptimal cerebral perfusion. In contrast, recordings during the use of the filming oxygenator with higher rates of flow have shown little or no deviation from the base line patterns of the anesthetized subject.

Our experience confirms the impression of Clowes and his associates[1] that prolonged isoelectric EEG tracings during and/or following bypass procedures are accompanied by poor chances for survival. No convulsive phenomena were observed during bypass with any of the oxygenating devices used in this study.

During bypass electroencephalographic activity which can fluctuate freely with changes in anesthetic concentrations is most desirable. This has been best accomplished in our experience by maintenance of rates of flow (70 to 120 c.c. per kilogram per minute) which are sufficient to allow arterial pressure to remain stable at 90 to 120 mm. Hg. Low flows, however well tolerated for short periods, apparently reduce tissue reserves and thereby increase the hazards of only momentary additional circulatory insults. EEG evidence of impaired cerebral function in our experience with the filming oxygenator has occurred only during the resumption of cardiac circulatory efforts immediately after conclusion of the bypass procedure. It is possible and important to correct electroencephalographic abnormalities promptly at this point by restoring adequate circulation either by return to the pump-oxygenator or by other means of support. In this connection the EEG becomes a valuable aid in "titrating" the physiologic tolerance to transitory periods of hypotension during resumption of cardiac circulatory efforts.

Since severe alterations in cortical electrical activity always occurred during bypass procedures when the microbubble and macrobubble oxygenators were

employed, restoration of base line EEG patterns was only possible by termination of extracorporeal circulation with resumption of adequate cardiopulmonary function by the subject. If defective cardiac activity existed immediately after bypass, serious cerebral damage could be expected.

Our EEG findings, when the filming oxygenator was used, parallel in most instances those reported by Theye and associates[10] from the Mayo Clinic. However, we have not observed the electroencephalographic changes which they noted immediately after extracorporeal circulation was instituted. These or similar changes were noted when the macrobubble device was employed in our studies and were attributed to the circulation of unoxygenated blood present in the tubing and defoaming chambers. In the filming device continuous oxygenation of reservoir blood prior to use of the extracorporeal circulation eliminates this defect.

The electroencephalograph obviously cannot pinpoint intrinsic deficiencies in an oxygenating and/or circulating unit. Although apparently accurately reflecting the adequacy of cerebral perfusion during bypass, it provides no definite assurance of the adequacy of perfusion of other vital organs. We, as well as others, have observed that large quantities of air injected into the carotid artery of dogs produce very minimal EEG changes, but death without recovery of consciousness commonly resulted. It would therefore be difficult to interpret the EEG changes encountered when the microbubble and macrobubble oxygenating devices were employed as evidence of cerebral air embolism. Experimental evidence by Maloney and his associates,[8] however, seems to indicate that air embolism is produced when the macrobubble device has been used.

Technical problems of EEG operation are markedly increased during employment of the pump-oxygenator because of the many necessary electrical devices and because of unavoidable motion. Shielding of the subject to eliminate 60 cycle recording interference is impossible. Therefore, all electrical equipment must be carefully grounded. Movement artifacts must be eliminated by readjustment of scalp electrodes when necessary and stabilization of all lead wires. These difficulties should not prevent effective employment of the EEG during operating room conditions.

This study has demonstrated that the nonspecific summation of the physiologic and metabolic processes as expressed by the electrical activity of the brain is an excellent gauge for judging from moment to moment the immediate state of neurologic viability of a subject during and after employment of a pump-oxygenator.

SUMMARY

1. Electroencephalographic observations have been made during cardiopulmonary bypass in 150 dogs and 23 patients using microbubble, macrobubble, and filming oxygenators.

2. With microbubble and macrobubble oxygenators undesirable alterations in EEG activity were observed consistently.

3. In contrast, recordings during bypass with the filming oxygenator have shown little deviation from the base line patterns of the anesthetized subject.

4. It is concluded that the EEG is a reliable and sensitive indicator of the adequacy of cerebral perfusion by oxygenated blood during bypass procedures with pump-oxygenators.

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DISCUSSION

DR. GEORGE H. A. CLOWES, JR. (Cleveland, Ohio).—Nobody knows what an electroencehalogram really is recording. We do know certain things that depress the potential that is recorded—namely, oxygen tension falling, which Dr. Bluer in our clinic has shown in the brain. We know from our own work that CO_2 tension rising will do the same thing. Dr. Maloney has shown that embolization does this. Our low flow group of dogs behaved in the same way, showing depression. However, Dr. Varco summarized the situation at the National Institute of Health meeting at Chicago this past fall and showed that his patients did not show much change, but that blood flow was regulated according to changes. If there was a depression, the blood flow was simply increased.

DR. RALPH A. DETERLING, JR. (New York, N. Y.).—Have you had an opportunity to use the evaluating device reported here with low flows and the filmer? I rather got the impression that this was to set up the filmer as far superior to the macrobubbler, yet the flows were quite different.

Which particular artery was used for perfusion? Have you had an opportunity to test the electroencephalographic changes with the subclavian artery compared to the femoral? Some authors have stated that marked differences do occur there.

Regarding the effect of air embolization, particularly in dogs, we were quite concerned about it when a significant air embolization occurred in 2 patients and, although the neurologic picture was extremely grim, including electroencephalographic evaluation, the recovery of both patients has been extremely good with very fine deficits remaining as of some months later, but these are improving.

DR. KARL E. KARLSON (Brooklyn, N. Y.).—Our experiments in regard to the injection of oxygen or air into the internal carotid artery of the dog indicate that the electroencephalogram may change very little, if at all. Then the dog recovers, probably, and recovers slowly, but if you like, you may say that it is almost normal.

If one autopsies such an animal that has had only border line changes in electroencephalogram and clinical appearance, the gross findings in the brain 2 months later are very striking, indicating that a significant degree of brain damage has occurred, although the clinical evidences in the dog, in the experimental animal, are minimal.

DR. OWENS (Closing).—We have not studied the filming device at low flows. We have not perfused the subclavian artery with the filming device. However, during the studies with the micro and macro device, we used various sites of perfusion and no obvious differences were noted.

The subject of embolization brings up one subject that I must pass on briefly. In 2 of our patients who have expired following pumping procedures, I was curious to find out more about the colloid nature of the organs and did some fat stains on the brain, kidney, and lung. Surprisingly enough, a great amount of fat emboli was found in the glomeruli of the kidney and in the capillaries of the brain. We do not know what significance this has.

MECHANICAL SUPPORT OF THE CIRCULATION IN ACUTE HEART FAILURE

JOHN E. CONNOLLY, M.D.* (BY INVITATION), MARVIN B. BACANER, M.D. (BY INVITATION), DAVID L. BRUNS, M.D. (BY INVITATION), JEROLD M. LOWENSTEIN, M.D. (BY INVITATION), AND EDGAR STORLI, M.D. (BY INVITATION), SAN FRANCISCO, CALIF.

(From the Department of Surgery, Stanford University School of Medicine)

THE successful introduction of various pump-oxygenators has opened the field of direct vision intracardiac surgery. However, there may well be an even greater horizon for cardiopulmonary bypass, namely, the treatment of nonsurgical conditions such as acute heart failure and massive pulmonary embolism. The possibilities of such an approach are not unique with us and indeed several groups have employed pump-ogygenators in the treatment of patients with myocardial infarction and pulmonary insufficiency.[6, 11]

However, the successful treatment of a patient with acute heart failure may involve support for periods of hours or even days of intermittent treatment. Although a number of pump-oxygenators have been performing well for intracardiac surgery, they are at present fraught with difficulties if the perfusion is carried beyond an hour or two. Also the operation of a bypass on a pump-oxygenator is a large and expensive undertaking involving considerable personnel.

Two therapeutic adjuncts in the treatment of heart failure, namely, venesection for right heart failure and intra-arterial transfusion in myocardial infarction, have enjoyed popularity from time to time.[1] Neither of these tools can be employed continuously for any length of time, the former because of the resultant depletion of blood volume with ensuing shock and the latter because of overloading of the circulation. It seemed reasonable to try a combination of the two, a continuous venesection with replacement of this venous blood on the arterial side. We thought that such a bypass might relieve the right-sided congestion in right heart failure, and the replacement of this blood into the arterial system might help rectify the hypotension that accompanies severe right-sided failure. In view of the reports of transient improvement following intra-arterial transfusion for myocardial infarction, we thought perhaps perfusion of the coronaries in a continuous fashion by a pump bypass would also restore the decompensated left ventricle. We employed the following experiments to test these hypotheses.

Supported in part by the Life Insurance Medical Research Fund.

Read at the Annual Meeting of the Society of University Surgeons, Boston, Mass., Feb. 13-15, 1958.

*John and Mary Markle Foundation Scholar.

METHODS

Healthy adult mongrel dogs were used and anesthetized with Nembutal. Plastic cannulae for a bypass were inserted through a groin incision into the inferior vena cava and abdominal aorta via the femoral vessels. The bypass consisted of a circuit of plastic tubing connected to the vena cava and aortic catheters via a reservoir and pump (Fig. 1). A Sigmamotor pump was employed in our experiments. Blood pressures were recorded from the opposite femoral artery, the right ventricle, and aortic arch through catheters by means of Strathan strain gauges and a Brush recorder. Jugular venous pressure was measured through a catheter connected to a water manometer.

A plastic adjustable band was placed around the main pulmonary artery trunk for purposes of controlled constriction.[8] The thoracic aorta just distal to the left subclavian artery was freed from surrounding tissues so it could be constricted or totally occluded. Cardiac outputs were determined by a technique devised by one of us (J. L.) which is similar to those of Huff and associates[3] and Veall and co-workers.[12] A scintillation counter with a lead collimator was placed above the heart and a graphic record of counts was obtained. Five cubic centimeters of I^{131}-tagged Rose Bengal (1 μc/c.c.) was injected into the right ventricle through a catheter for each determination. It was not necessary to obtain repeated blood samples with this method.

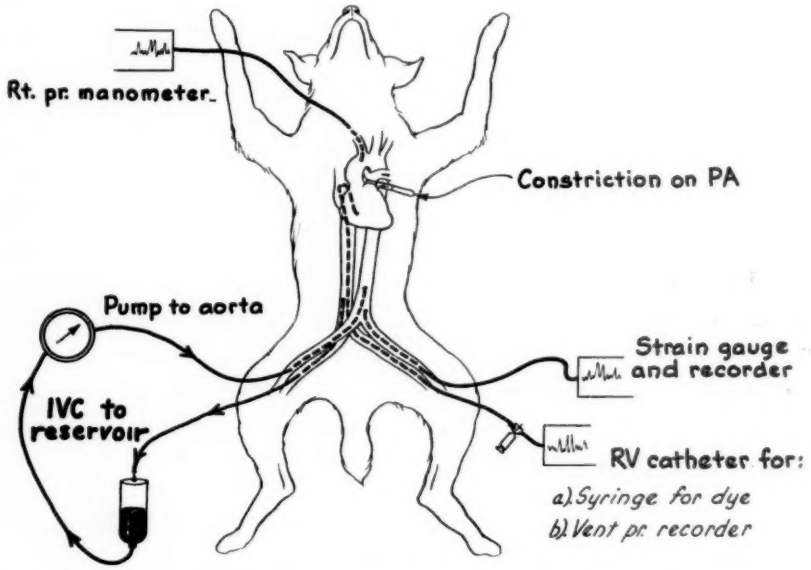

Fig. 1.—Diagram of experimental preparation. Bypass is situated on right.

The following study was devised to test the efficacy of the bypass on experimental failure. Failure of the right heart was induced by constriction of the pulmonary artery 22 times on 9 dogs. Several base line cardiac outputs were determined. Then graded constriction of the pulmonary artery was carried out until there was slowing, irregularity, and dilatation of the heart, and asystole was imminent. At this point another cardiac output was taken and the effects of (1) bleeding from the venous side alone, (2) transfusing the venous side alone, and (3) employing the vein-to-artery bypass on cardiac output, systemic pressure, and right ventricular action were studied. The bypass was run for periods of 2 to 17 minutes. In addition to the pump bypass, 3 experiments were performed in which the blood in the reservoir was returned manually using a hand bulb attached to an intravenous flask.

Two survival experiments were performed to test the effects of long-term femoral vein-to-aorta bypass. Roughly 15 per cent of the calculated resting cardiac output was bypassed for periods of 8 and 12 hours. Venous blood samples were withdrawn at 2- and 4-hour intervals, respectively, for platelet counts.

RESULTS

As can be seen by referring to Tables I through V, the amount of pulmonary artery constriction employed throughout all experiments resulted in negligible cardiac outputs and systemic pressures of 0 to 30 mm. Hg.

Effects of Venous-to-Arterial Bypass on Cardiac Failure Induced by Pulmonary Artery Constriction.—As can be seen from Table I, outputs taken after the bypass had been in operation from 2 to 17 minutes, ranged from 0.2 to 1.2 blood volumes per minute, representing a significant increment from pre-bypass levels in all cases. From Table I it can be seen also that in all experiments except Dog 6, Dog 5 (d), and Dog 9, the cardiac output fell to zero or an insignificant level when the bypass stopped. When the bypass was restarted the cardiac output again increased. Fig. 2 demonstrates graphically the tracings

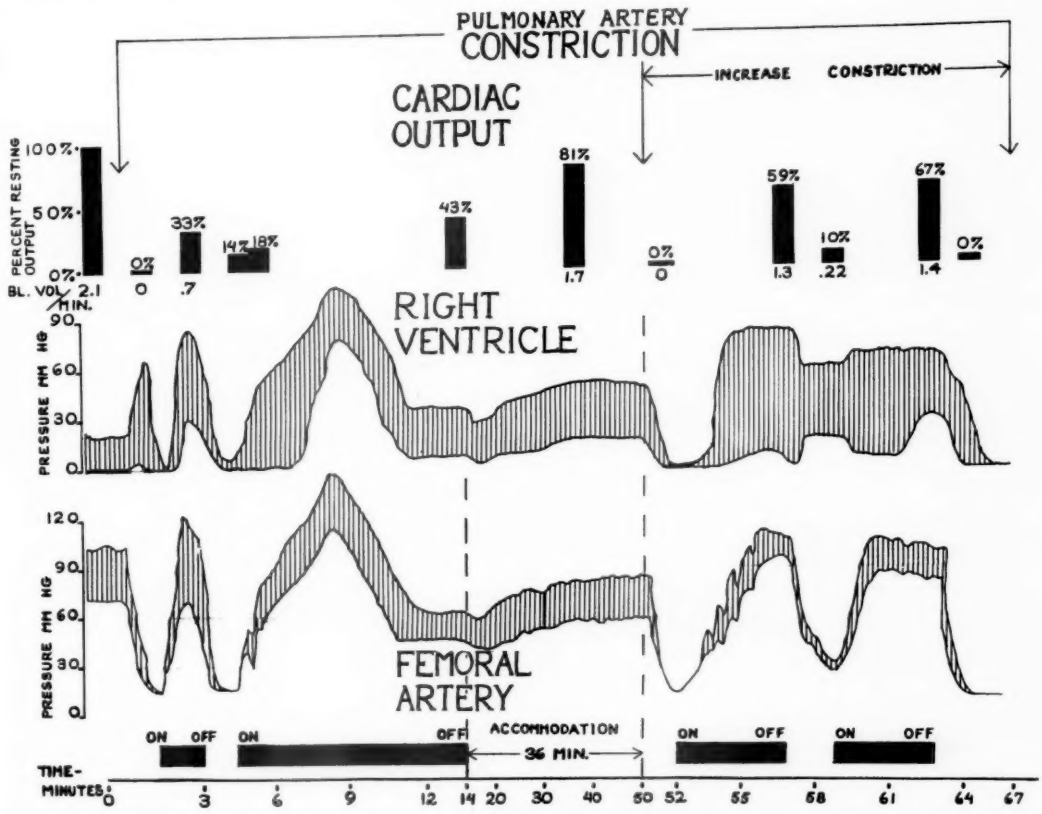

Fig. 2.—(Dog 2.) Graphic record of the effects of pulmonary artery constriction and bypass pumping on cardiac output and on right ventricular and femoral artery pressures. The dark blocks just above the time scale represent bypass pumping in operation.

of peripheral pressure, right ventricular pressure, and cardiac output in a typical experiment on Dog 2. It can be seen here that the systemic and ventricular pressures rose concomitantly with the increases in the cardiac output whenever the bypass was in operation. The systemic and ventricular pressures remained elevated above base line constriction levels after the pump had been stopped. This is one of the 3 instances of accommodation encountered after the bypass had been discontinued.

TABLE I. CARDIAC OUTPUTS WHEN PULMONARY ARTERY WAS CONSTRICTED FOLLOWED BY VENOUS-TO-ARTERIAL BYPASS

DOG	RUN	RESTING CARDIAC OUTPUT (BL. VOL. MIN.)	PULMONARY ARTERY CONSTRICTION CARDIAC OUTPUT	CARDIAC OUTPUT BYPASS ON (MINUTES)	BYPASS OFF	BYPASS ON
1		2.5	0.16	0.54 (17)	0.26	
2	a	2.1	0.0	0.21 (14)	Too much constriction	
	b		0.0	0.90	0.30	0.38
	c		0.0	1.3 (5)	0.22	1.4
3	a	2.0	0.0	0.64 (5)		
	b		0.14	0.65 (3)		
	c		0.2	0.45 (2½)		
	d		0.18	0.78 (7½)	0.12	0.72
4	a	2.0	0.0	1.3 (5)		
	b		0.43	0.61 (2)		
	c		0.80	1.0 (4)	0.0	0.46
5	a	2.3	0.55	0.82		
	b		0.53	0.88		
	c		0.30	0.44	0.15	
	d	2.0	0.0	1.2 (3)	0.66 (accommodated)	
6	a	1.6	0.51	1.1 (4)	1.2 (accommodated)	
	b	1.2	0.09	0.43 (3)		
7		1.72	0.0	0.320 (hand pumping on arterial side)		
8	a	2.1	0.0	0.510 (hand pumping on arterial side)		
	b	1.7	0.0	0.420 (hand pumping on arterial side)		
9	a	2.4	0.4	1.23 (5)	0.86 (accommodated)	
	b		0.0	0.625		

After accommodation in this dog, further constriction of the pulmonary artery dropped cardiac output and pressures to zero. The reinstatement of the pump bypass again raised pressures and output but accommodation did not occur when the bypass was once more discontinued.

Effects of Venesection Alone With Pulmonary Artery Constriction.— Removal of blood from the vena cava further decreased cardiac output and

TABLE II. CARDIAC OUTPUTS WITH VENESECTION AND FIXED PULMONARY ARTERY CONSTRICTION

DOG	RUN	RESTING CARDIAC OUTPUT	PULMONARY ARTERY CONSTRICTION CARDIAC OUTPUT	BLEEDING FROM VENA CAVA	PUMP ON AND LENGTH OF TIME	OFF	ON
5	a	2.3	0.55	0.29	0.82		
	b		0.53	0.30	0.88		
	c		0.30	0.0	0.44	0.15	
	d	2.0	0.0	0.0	1.2	0.66	accommodated
6	a	1.6	0.51	0.0	1.1	1.2	accommodated

systemic pressure in 5 experiments on 2 dogs, whereas the subsequent institution of partial bypass significantly raised cardiac output in the presence of a fixed amount of pulmonary artery constriction.

TABLE III. EXPERIMENT WITH VENOUS OVERLOADING IN ADDITION TO PULMONARY ARTERY CONSTRICTION

DOG	RESTING CARDIAC OUTPUT	PULMONARY ARTERY CONSTRICTION CARDIAC OUTPUT	CARDIAC OUTPUT AFTER RAPID INFUSION OF 200 ML. BLOOD VIA JUGULAR VEIN
13	1.9	0	0.17

Effect of Venous Overloading With Pulmonary Artery Constriction.—As can be seen in Table III, in the presence of fixed pulmonary artery constriction, cardiac output did not rise appreciably with a venous transfusion of 200 c.c.

Because these results seemed to show some relationship between the root pressure attained and cardiac function, we decided to test cross-clamping of the aorta in place of the pump bypass. In 3 experiments on 2 dogs, the aorta was cross-clamped distal to the left subclavian artery after experimental failure had been produced by pulmonary artery constriction. In a final experiment, the aorta was cross-clamped distal to the left subclavian artery without pulmonary artery constriction and at various periods after aortic cross-clamping.

Effects of Cross-Clamping of the Aorta in Addition to Pulmonary Artery Constriction.—Table IV shows that cardiac output was improved two- or three-fold when the aorta was cross-clamped just distal to the left subclavian artery in the presence of a fixed marked constriction of the pulmonary artery. The root pressure was elevated to a mean pressure of 100 or over in each of these 3 experiments by the aortic cross-clamping. Release of the cross-clamping dropped the cardiac output to its former level and reclamping it again raised the cardiac output (Fig. 3).

TABLE IV. EXPERIMENTS IN WHICH IN ADDITION TO FIXED PULMONARY ARTERY CONSTRICTION THE AORTA WAS CROSS-CLAMPED

DOG	RUN	RESTING CARDIAC OUTPUT	PULMONARY ARTERY CONSTRICTION CARDIAC OUTPUT	CARDIAC OUTPUT WITH AORTIC CROSS-CLAMPING	CARDIAC OUTPUT AFTER RELEASE OF AORTA	CARDIAC OUTPUT WITH RECLAMPING OF AORTA
9	a	2.4	0.360	0.950	0.41	
	c	1.4	0.270	0.600 (root pressure 100)	0.0	0.500
10		1.15	0.20 (root pressure 55/35) (right ventricular pressure 35/9)	0.690 (root pressure 155/130) (right ventricular pressure 75/9)	0.180	0.330

Effects of Cross-Clamping of the Aorta Without Pulmonary Artery Constriction.—In two experiments performed, the root pressure rose in both while the cardiac output at first did not change and later fell in spite of sustained high root pressures (Table V).

TABLE V. EXPERIMENTS IN WHICH THE AORTA WAS CROSS-CLAMPED WITHOUT PULMONARY ARTERY CONSTRICTION

DOG	RESTING CARDIAC OUTPUT	CARDIAC OUTPUT AND AORTIC ROOT PRESSURE AFTER CROSS-CLAMPING (MINUTES) 1	5	15	30	45	60	AFTER RELEASE
13	1.9	1.9 155/120 root		0.90 165/130	0.89 170/150	0.55 80/30	0.50 65/50	1.0 60/45
14	0.78	0.75	0.75					

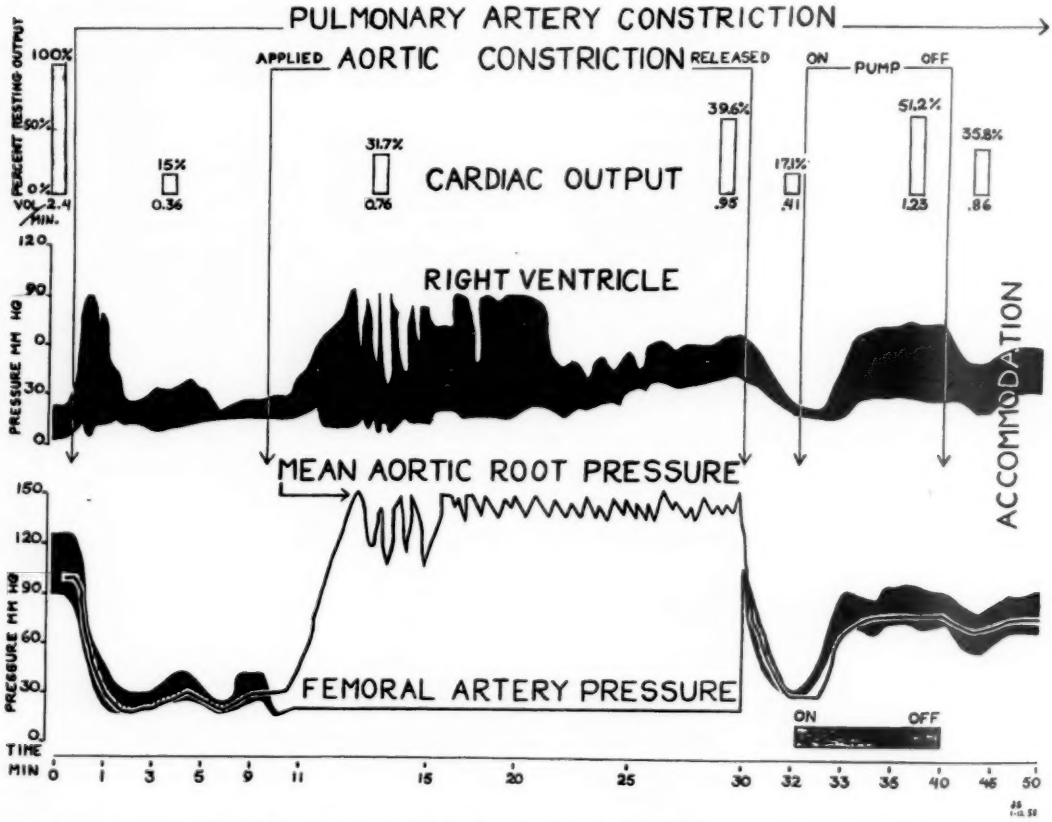

Fig. 3.—(Dog 9.) Graphic record of the effects of aortic cross-clamping after pulmonary artery constriction. The response to subsequent bypass pumping is shown.

Effects on Platelets of Long-Term Partial Bypass of Unoxygenated Blood.—Table VI shows the progressive linear fall in circulating platelets during a prolonged bypass. It should also be noted that frequent doses of heparin were required in our bypass of unoxygenated blood, as opposed to a bypass with a pump-oxygenator, presumably because the artificial oxygenation process destroys many more platelets than the simple pumping of blood. Both animals survived and recovered without evident ill effects.

TABLE VI. EFFECTS OF PROLONGED BYPASS ON CIRCULATING PLATELETS

DOG	0	2 HR.	4 HR.	6 HR.	8 HR.	10 HR.	12 HR.
15	422	264	174	160*	110*		
16	265		205		170		155*

Platelets $\times 10^3$.
*Clumping.

DISCUSSION

Thus in our experiments we have used pulmonary artery constriction to cause acute cardiac failure and in turn have tested the effect of a partial venous-to-arterial shunt under pressure, on the ability of the heart to perform work as reflected by its output and pressure. In all cases, in spite of a constant fixed pulmonary artery constriction, the cardiac output, systemic pressure, and ventricular contractions increased with the pump bypass only to fall again when the pumping was terminated.

The fact that cardiac work output increased when the aortic root pressure was raised by the bypass or by cross-clamping of the aorta suggests that the improvement may be due to an increase in coronary flow. This is further substantiated by the experiments of Salisbury[7] who concluded that augmented coronary artery pressure would cause a remarkable increment of the right ventricular strength. Furthermore, it would also appear that raising the root pressure aided the heart only when it was placed under a burden as evidenced by the improvement with cross-clamping of the aorta after pulmonary artery constriction whereas the output remained the same or fell when cross-clamping was not accompanied by pulmonary artery constriction. Our results would suggest that the critical root pressure for adequate coronary perfusion, and in turn myocardial work ability, is 80 mm. Hg. It is of interest that in spite of the lowered saturation of the aortic root blood during shunting that the myocardium was able to extract enough oxygen to carry out work.

The use of l-noradrenaline in the treatment of shock secondary to myocardial infarction and pulmonary embolism is now fairly well established.[2, 5, 9, 10, 13] However, many patients do not respond to its use and the employment of a partial venous-to-arterial shunt with pressure perfusion of the coronaries might be expected to provide lifesaving support for periods of time in which reversibility or accommodation to the underlying pathology could occur. Periods of partial bypass in the dog were well tolerated for 8 and 12 hours and likely for indefinite periods. In view of the tendency for accommodation in the dog after short periods of bypass, the possibility of intermittent bypass during the critical hours or days of failure is suggested.

The technique of bypass in a properly selected patient would consist of a small groin incision with exposure of the femoral artery and vein. A plastic catheter would be passed through each of these vessels into the terminal aorta and vena cava, respectively. The venous catheter would drain into the reservoir when it would be pumped back to the arterial system. Only one unit of blood is necessary for priming this system and heparin (2 mg. per kilogram) is given

by vein just before placing the catheters. The entire bypass could be in operation in 20 minutes and could be carried out in the emergency room or at the bedside.

SUMMARY

1. A simple femoral venous-to-arterial bypass of unoxygenated blood has been devised to provide support of the circulation in acute heart failure.

2. In experimental animals subjected to severe cardiac stress, its employment has resulted in a remarkable restoration of myocardial strength.

3. The possible role of the coronary circulation in producing these results is discussed.

4. Several clinical applications are suggested.

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Review of Recent Meeting

MEETING OF THE SOCIETY OF UNIVERSITY SURGEONS

FEBRUARY 13-15, 1958, BOSTON, MASS.

VICTOR RICHARDS, M.D., SAN FRANCISCO, CALIF.

The nineteenth annual meeting of the Society of University Surgeons was held in Boston, Massachusetts, on Feb. 13, 14, and 15, 1958, where the Society was the guest of the Harvard Medical School.

On Thursday morning, February 13, the members and an unusually large number of guests assembled in Amphitheater D of Harvard Medical School where the official meeting was opened by Dr. Richard Warren, with Dr. Oliver Cope and Mr. McGeorge Bundy serving as speakers. The Society was officially welcomed by Drs. Richard Warren, Oliver Cope, and Mr. McGeorge Bundy.

Representatives of several of the major hospitals of the Boston area joined together to offer the entire program on Feb. 13, 1958. The morning session consisted of a Symposium on Blood Flow and Related Problems and the following papers were given:

1. Oxygen Supply to the Renal Tubules—Dr. John R. Pappenheimer.
2. Pulmonary Blood Flow—Dr. James L. Whittenberger.
3. Coronary Collateral Flow Studies—Dr. Howard A. Frank, Dr. Peter Lambert, and Dr. Sven Bellman.
4. Perfusion Rates, Biochemical and Clinical Results in Extracorporeal Circulation for Children—Dr. Lester R. Sauvage.
5. Liver Blood Flow in the Dog—Dr. William C. Shoemaker, Dr. Louis I. Smith, and Dr. Richard W. Steenburg.
6. Some Observations on Hepatic Flow in Man—Dr. George L. Nardi, and Dr. Hugo M. Palazzi.
7. The Effects of Hemorrhage on Adrenal Blood Flow and Hormone Output in the Dog—Dr. Willia F. Walker.
8. Superior Mesenteric Arterial Insufficiency—Dr. Robert S. Shaw, Dr. Edward P. Maynard, Dr. W. Hardy Hendren III, and Dr. Donald Glotzer.
9. The Effect of High and Low Viscosity on Capillary Flow—Dr. Lars-Erik Gelin.

At noon, lunch was served at the Sheraton-Plaza Hotel and in the afternoon in the Main Ballroom of the Sheraton-Plaza Hotel, 2 symposia were offered: (1) a Symposium on Transplantation of Tissue and Organs, and (2) a Symposium on Metabolic Studies. Dr. Moore presided at the Symposium on Transplantation of Tissue and Organs. The following papers were given:

1. Kidney Transplantation Between Identical Twins: Indications and Surgical Treatment—Dr. Joseph E. Murray and Dr. J. Hartwell Harrison.
2. Current Approaches to the Study of Homografts—Dr. John P. Merrill.
3. Biochemical and Functional Studies in Extracorporeal Kidney Perfusion—Dr. Nathan Couch.
4. Transplantation of Bone Marrow; the Sex Leukocyte Tag—Dr. Kendrick A. Porter.
5. Induction and Transplantation of Hormone Responsive Tumors—Dr. Jacob Furth.
6. Experience With Experimental Adrenal Cortical Transplantation—Dr. Paul S. Russell and Prof. P. B. Medawar.

7. Endocrine Tissue Transplantation Using the Millipore Membrane—Dr. John R. Brooks, Dr. Albert C. G. E. de Scoville, and Dr. Julio C. Priario.
8. Recent Progress in the Transplantation of Human Tumors. Dr. W. Bradford Patterson.

Dr. William V. McDermott, Jr., presided at the Symposium on Metabolic Studies. The following papers were given:

1. Comparison of Simultaneous Distribution of Mg^{28}, K^{42}, and Ca^{45}—Dr. Benjamin A. Barnes, Dr. Gordon Brownell, and Dr. Oliver Cope.
2. Molecular Configuration of Collagen Fibrils and Its Relation to Calcification—Dr. Melvin J. Glimcher.
3. A Rate Limiting and Vulnerable Step in Urea Synthesis—Mr. Don C. Pearl, Jr., and Dr. William V. McDermott, Jr.

A very pleasant cocktail party and dinner dance concluded the opening day of the meeting. These festivities were held at the Sheraton-Plaza Hotel.

The Scientific Program presented by members of the Society on February 14 and 15 is published in this issue of SURGERY.

Calendar of Surgical Meetings

AMERICAN ACADEMY OF NEUROLOGICAL SURGERY. *Annual Meeting,* Nov. 5-8, 1958, Royal York Hotel, Toronto, Ontario, Canada. Secretary, Dr. Robert McLaurin, University of Cincinnati, Cincinnati, Ohio.

AMERICAN ASSOCIATION FOR THE SURGERY OF TRAUMA. *Annual Meeting,* Oct. 2-4, 1958, The Drake Hotel, Chicago, Ill. Acting Secretary, Dr. James K. Stack, 700 N. Michigan Ave., Chicago 11, Ill.

AMERICAN CANCER SOCIETY. *Annual Meeting,* Oct. 20-21, 1958, Biltmore Hotel, New York City, N. Y. Secretary, Dr. Scott Hill. Subject, cancer of the colon and rectum.

AMERICAN COLLEGE OF SURGEONS. *Clinical Congress,* Oct. 6-10, 1958, The Conrad Hilton, Chicago, Ill. Secretary, Dr. Michael L. Mason, 40 E. Erie St., Chicago, Ill.

AMERICAN MEDICAL ASSOCIATION. *Annual Meeting,* Dec. 2-5, 1958, Leamington Hotel, Minneapolis, Minn. Deadline for submission of abstracts, Aug. 1, 1958. Secretary, Council on Scientific Assembly, Dr. Thomas G. Hull, 535 North Dearborn St., Chicago 10, Ill.

INTERNATIONAL COLLEGE OF SURGEONS. *Alabama Surgical Division Meeting,* Medical College of Alabama, Birmingham, Oct. 30-31, 1958. Regent of Alabama, Dr. Paul Wolfe Shannon, 1927 First Ave. North, Birmingham 3, Ala. *Mid-Atlantic Regional Meeting,* Homestead Spa, Hot Springs, Va., Nov. 17-18, 1958. Regent of Virginia, Dr. Elbyrne G. Gill, 711 Jefferson St. South, Roanoke 13, Va. *Fifth Annual Congress of the Japanese Section,* Tokyo, Japan, Oct. 17, 1958. For information write to the Secretariat, 1516 Lake Shore Drive, Chicago 10, Ill.

INTERNATIONAL UNION AGAINST CANCER. *Seventh International Cancer Congress,* July 6-12, 1958, London, England. Secretary General, Harold Dorn, National Institute of Health, Bethesda 14, Md.

NEW ENGLAND SURGICAL SOCIETY. *Annual Meeting,* Oct. 3 and 4, 1958, Mount Washington Hotel, Burton Woods, N. H. Deadline for submission of papers, June 1, 1958. Secretary, Dr. Richard Warren, Peter Bent Brigham Hospital, 721 Huntington Avenue, Boston 15, Mass.

SOCIETY OF UNIVERSITY SURGEONS. *Annual Meeting,* Feb. 12-14, 1959, Denver, Colo. Secretary, Dr. James D. Hardy, University of Mississippi Medical Center, Jackson, Miss.

SOUTHERN SURGICAL ASSOCIATION. *Annual Meeting,* Dec. 9-11, 1958, Boca Raton Hotel and Club, Boca Raton, Fla. Attendance by invitation of membership only. Secretary, Dr. George G. Finney, 2947 St. Paul St., Baltimore 18, Md.

WESTERN SURGICAL ASSOCIATION. *Annual Meeting,* Nov. 20-22, 1958, Hotel Kahler, Rochester, Minn. Deadline for submission of papers, Sept. 1, 1958. Secretary, John T. Reynolds, M.D., 612 N. Michigan Ave., Chicago 11, Ill.